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ANTIBODIES IN DOGS IN CENTRAL OHIO

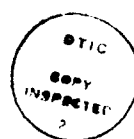
A Thesis

Presented in Partial Fulfillment of the Requirements  
for the Degree Master of Science

by

Howard L. Pue, BS, DVM

The Ohio State University  
1983



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## I. Introduction

Canine brucellosis, caused by the bacterium Brucella canis, is an insidious disease which is difficult to diagnose. It is characterized by abortion in the female dog, and infection of the genital tract of the male. The subsequent loss of reproductive capacity, and the extremely contagious nature of the disease among dogs have resulted in devastating economic and personal losses to many dog owners, particularly breeders of pet and research animals. No effective vaccine exists for the prevention of canine brucellosis, and treatment of the disease is very unreliable. Control of this disease can only be attained by detection and segregation of infected animals. Brucella canis is transmissible from dogs to humans. Such infections have been reported as laboratory acquired as well as the result of handling infected animals.

The purpose of this study was to determine the prevalence of Brucella canis-infected dogs in select canine populations by the 2-mercaptoethanol tube agglutination test. Analysis of prevalence figures with respect to demographic data (e.g., stray/non-stray, male/female) was accomplished. In addition, the serologic technique employed in the canine brucellosis serosurvey was further tested to determine its comparative effectiveness when using hemolyzed versus nonhemolyzed serum samples, and when using plasma versus serum samples.

Furthermore, the results of two other serologic techniques were compared with those of the 2-mercaptoethanol tube agglutination test, in an attempt to ascertain the ability of each test to detect Brucella canis serum agglutinins. Animals identified during the course of this

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cont'd

study as having serologic evidence of Brucella canis infection were subjected to follow up study when possible (i.e., additional serologic testing, blood and tissue culture, pathologic examination, et cetera).

## II. Literature Review

### Historical Aspects

Between January, 1964 and April, 1967, a marked increase in abortions occurred in a research canine breeding colony operated jointly by the Texas Department of Corrections and the Baylor College of Medicine<sup>40,58</sup>. This colony, originally established in 1962, consisted of beagles, greyhounds, and pointers, and had a population of breeding females varying from a high of 68 to a low of 32. Although the initial abortion in January, 1964 occurred in a beagle, all breeds in the colony eventually experienced abortion. Isolates obtained from tissues of aborted fetuses and peripheral blood of bitches yielded a small, Gram-negative bacterium. Biochemical, cultural and serological characteristics indicated that the microorganism was most similar to members of the genus Brucella. During the years 1965 and 1966, bacterial abortion was diagnosed in four kennels in four counties of South Carolina<sup>89</sup>. About 75 adult female beagles were involved in these epidemics. An unclassified Gram-negative coccobacillary bacterium (biochemically and antigenically related to genera of the family Brucellaceae) was isolated from fetal tissues and vaginal discharges of involved animals. From June, 1966 to October, 1966, over 200 abortions were reported to the Veterinary Virus Research Institute, Cornell University, Ithaca, New York<sup>7</sup>. These abortion episodes occurred in 13 states, representing all regions of the country. The only breed involved was the beagle, and again, a Gram-negative coccobacillus was isolated. Cultural, biochemical and serologic studies placed the organism in the family



Brucellaceae. Virologic examinations for distemper, infectious canine hepatitis, reovirus-type 1, and canine herpesvirus were negative. In 1967, investigators at the College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, described the principle characteristics of an unclassified, Gram-negative bacterium associated with an infection of bitches which frequently resulted in either abortion, stillbirths, or neonatal death<sup>62</sup>. Isolates of the bacterium were obtained from the infected tissues of 27 fetuses or neonates and from vaginal swabs derived from 17 bitches. The canine breeds involved were not stipulated. As in the previous studies, taxonomical placement of the isolated bacterium was in the family Brucellaceae, and it was noted that this particular organism had been tentatively referred to as "Brucella canis". A severe abortion outbreak in a kennel in the midwestern United States beginning in January, 1968, was investigated by researchers from the University of Minnesota Medical School, Minneapolis, Minnesota<sup>66</sup>. The kennel population consisted of 178 beagles, all of which were adults except for 37 puppies three months of age or less. There was a ratio of one adult male for every four adult females. Abortions occurred from January, 1968 to January, 1969, reaching a peak in June, 1968. Before the epidemic was brought under control, at least 41 females had aborted, and 86 % (121/141) of the adult beagles were shown to be infected by serological and bacteriological testing. Tissues from 13 aborted and stillborn fetuses were examined, and the organism tentatively classified as Brucella canis was isolated from all of them.

Numerous studies were conducted in an effort to establish the taxonomic position of Brucella canis. Research data published in 1968<sup>51</sup>

was based on cultural growth characteristics, serological and toxicity testing, and electron microscopy. The authors concluded that the agent in question was of the Brucella genus, and was a "new" species (i.e., Brucella canis) rather than a biotype of a previously identified species of Brucella. Also in 1968, researchers from the University of Wisconsin, Madison, Wisconsin<sup>21</sup>, published the results of antigenic testing (agglutination, agglutinin-absorption, immunoelectrophoresis, and gel diffusion tests), in which they also concluded that the agent of canine abortion should be classified as Brucella canis. The same year, Carmichael and Bruner<sup>10</sup>, on the basis of morphological, cultural, biochemical, and serological comparisons, as well as pathogenicity and gas chromatographic studies, suggested the name of Brucella canis for the bacterium in question. Again in 1968, researchers at the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland<sup>46</sup>, conducting deoxyribonucleic acid homology studies, concluded that the agent of canine abortion was a member of the genus Brucella. In light of these and other similar results, Brucella canis was accepted as a species in 1970 by the International Committee on Nomenclature of Bacteria, Subcommittee of Taxonomy of Brucella<sup>8</sup>.

#### Causal Organism

It has been recognized for some time that each species of Brucella has a decided host preference<sup>59</sup>. That is, Brucella abortus for cattle, Brucella melitensis for sheep and goats, Brucella suis for swine, Brucella neotome for the wood rat, and Brucella canis for canines<sup>34</sup>.

While there is not total specificity between these species of Brucella

and their associated hosts, transmission from the preferential host to dissimilar hosts does not readily occur. Furthermore, when a Brucella species does produce disease in a dissimilar host, the organisms usually localize in the mammary gland and the reticuloendothelial system rather than in the uterus and fetal membranes<sup>59</sup>.

The Brucella canis organism is a small rod-shaped cell 0.5 by 0.5 to 2 microns in size<sup>10,58,62</sup>, occurring singly, in short chains, or in groups<sup>10,58</sup>. Fresh isolates tend to be coccoid in shape; however, bacillary forms occur after several transfers on artificial media<sup>10</sup>. The Brucella canis organism is thus often referred to as a coccobacillary bacterium<sup>26,58,66</sup>. The organism is nonmotile<sup>10,58,62</sup>, Gram-negative<sup>10,26,58,62,66</sup>, has only a limited cell wall<sup>66</sup>, and is encapsulated<sup>10,62</sup>. Brucella canis grows readily on tryptose agar<sup>10,58,62</sup> or Brucella agar<sup>10,66</sup> under aerobic conditions<sup>10,58,62,66</sup>, forming non-smooth ("rough") mucoid colonies<sup>10,26,58,66</sup>. The colonies are circular and convex<sup>58,62</sup>. Growth is inhibited by ten per cent carbon dioxide, and does not occur at all under strict anaerobic conditions<sup>10,62</sup>. Colonies become visible to the unaided eye after 36-48 hours<sup>10,62</sup>. After 3-4 days of incubation at the optimum temperature of 37° C<sup>10,62</sup>, and under other favorable conditions, colonies range in diameter from 0.3 to 2 mm.<sup>10,58,62</sup>, appearing grayish-white grossly<sup>10,58</sup>, with a blue-green opalescence when viewed microscopically under obliquely transmitted light<sup>10,58</sup>. In a liquid medium (e.g., tryptose broth), a viscous, ropy growth occurs after 48-72 hours<sup>10,26,66</sup>. There is no hemolysis of blood broth<sup>10</sup> or blood agar<sup>62</sup>.

Biochemical properties of Brucella canis include the following:

catalase production ..... positive<sup>10,26,58,62</sup>  
carbohydrate fermentation ..... negative  
urease production ..... positive  
indol production ..... negative<sup>10,58,62</sup>  
citrate utilization ..... negative  
oxidase production ..... negative<sup>10,26,62</sup>  
litmus milk ..... alkaline<sup>10,62</sup>  
nitrate reduction ..... positive  
growth on MacConkey's medium ..... negative

In their biochemical studies, Carmichael and Bruner<sup>10</sup> utilized eight isolates from aborted fetuses from different regions of the United States. They also used a reference strain of Brucella canis (RM-666) which Carmichael had earlier deposited with the American Type Culture Collection, and which was later (1970) designated as the reference strain by the Subcommittee of Taxonomy of Brucella. Additional biochemical properties noted by these researchers include:

hydrogen sulfide ... positive after one week  
oxidase production (with 1 % dimethyl-paraphenylene-diamine)..  
... positive  
gelatin liquifaction ... negative  
growth on medium containing basic fuchsin - 1:50,000 ....  
...negative; 1:100,000 ... positive  
growth on medium containing thionin - 1:25,000 ... positive;  
1:50,000 ... positive

Moore and Bennett<sup>62</sup> utilized up to 32 isolates obtained from affected fetuses and bitches in the determination of biochemical properties. Besides those listed above, these authors observed no hydrogen sulfide production in any of 32 isolates tested. However, the time period of observation was not stipulated in their subsequent publication, and it is possible that positive reactions would have occurred after a period of one week, as reported by Carmichael and Bruner<sup>10</sup>. McCormick et al.<sup>58</sup> utilized four isolates of Brucella canis derived from female dogs housed in a canine production colony plus the reference strain RM-666, in the determination of biochemical properties. In addition to properties shown above, these researchers reported inconsistent results for their isolates with respect to hydrogen sulfide production. Also, McCormick et al.<sup>58</sup> observed a lack of sensitivity of their isolates to thionin-containing media at concentrations of 1:25,000, 1:50,000 and 1:100,000 (this agrees with the results of Carmichael and Bruner<sup>10</sup>). However, McCormick et al.<sup>58</sup> found three of their four isolates to be sensitive (negative growth) to basic fuchsin at a concentration of 1:100,000 (not consistent with Carmichael and Bruner<sup>10</sup>). Flores-Castro et al.<sup>26</sup> utilized seven Mexican isolates plus strain RM-666 in their biochemical studies. Besides the data shown above, these researchers reported their isolates of Brucella canis to be negative for hydrogen sulfide production. Also, differences were observed between the reference strain (RM-666) and some of the Mexican strains with regard to nitrate reduction and dye-sensitivity characteristics. For example, three Mexican strains failed to reduce nitrate, and one Mexican strain was found unusually active.

A final property of Brucella canis, one which makes it unique among the Brucella species, is the fact that Brucella canis contains little or no somatic O antigen, indicating that the limited cell wall has only minimal amounts of endotoxin<sup>66,86</sup>. It has been suggested that the lack of endotoxemia in infected animals explains the absence of fever as well as the relative paucity of systemic clinical manifestations<sup>86</sup>.

#### "Typical" Clinical Features In The Dog

In general, dogs with canine brucellosis show few serious signs of systemic illness, and there is virtually no mortality<sup>11,12,66</sup>. This is in contrast with the severe manifestations of brucellosis often seen in cattle, swine, goats, and humans due to infections with other Brucella species. Usually the only sign of canine brucellosis in bitches reported by owners is spontaneous abortion without premonitory signs between days 30 and 57 of gestation<sup>11</sup>. Although owners occasionally report depression in bitches following abortion, usually the animals appear healthy prior to and following the abortion<sup>11</sup>. Since abortion is the predominant sign in females, canine brucellosis is not clinically apparent in the nongravid female<sup>12</sup>, especially to the owner or other untrained person. In one laboratory setting<sup>11</sup>, 85 % (325/385) of the experimentally infected bitches aborted between gestation days 45 and 55, with the most common time at about 50 days. In another experimental situation<sup>40</sup>, the average day of pregnancy on which abortion occurred was day 53, with a range of from day 49 to day 59. It has been experimentally observed that some bitches may abort or give birth to stillborn

or weak pups two or three times, whereas other bitches may whelp normal litters following a mating subsequent to a single abortion<sup>12,40</sup>. Another clinical feature sometimes observed is failure to conceive after one or more matings<sup>11,12,40,66</sup>. Such instances may be explained by early, undetected embryonic death (10-20 days after mating)<sup>11,12,40</sup>, or by bitches ingesting aborted placental tissues and fetuses (unobserved by the owner)<sup>11,12</sup>. Prolonged vaginal discharge following abortion is often seen in both experimentally and naturally infected bitches<sup>40,66</sup>. The period of discharge may range from 1-6 weeks<sup>11,12,40</sup>. Although the amount, color, and consistency of the discharge varies greatly, it usually appears serosanguineous in nature<sup>11,12</sup>. Retention of the placenta does not appear to be a problem in either natural or experimental situations<sup>11</sup>.

Canine brucellosis in male dogs is usually characterized by epididymitis<sup>11,12,26,33,65,66</sup>, scrotal dermatitis<sup>11,12,99</sup>, and prostatitis<sup>12,33,65,66</sup>, in both naturally and experimentally infected animals. The scrotal dermatitis is thought to be caused principally by Staphylococcus organisms that have invaded the moist scrotum, probably as a consequence of persistent licking of the area over painful epididymides<sup>12</sup>. Inflammation of the testicles is not a consistently described feature in most cases of canine brucellosis<sup>11,66</sup>. However, orchitis due to Brucella canis infection is known to occur<sup>12,33,65,99</sup>. One case report of orchitis<sup>82</sup> involved a two-year-old male Irish setter, clinically normal except for an enlarged scrotum, with viscous red-brown opaque fluid draining from ulcers in the scrotum. Serological tests showed an agglutination titer to Brucella canis of 1:200 or greater, and Brucella canis

was isolated from the draining ulcers in the scrotum. One testicle, although normal grossly, was found microscopically to be infiltrated by inflammatory cells. The other testicle was found to be entirely necrotic. Although this finding remained unexplained, possible causes included torsion of the spermatic cord, thrombosis of critical vessels, or necrosis of vessel walls. Acute and chronic necrotizing arteritis and phlebitis have been reported to occur in the prostate, scrotum, sheath, and other tissues of some dogs with canine brucellosis<sup>12</sup>. Although orchitis per se is not a consistent feature in infected males, testicular degeneration and atrophy<sup>11,12,65,66,99</sup> often occur, unilaterally or bilaterally, both naturally and experimentally. Affected males exhibit a painful response upon palpation of the testis or epididymis, except when atrophy has already occurred<sup>11</sup>. While males with bilateral testicular atrophy are usually sterile, unilateral testicular atrophy may or may not result in a sexually dysfunctional male. Depending on the individual, some males with unilateral testicular atrophy may be fertile and produce normal pups, while others may suffer from a loss of libido and/or be incapable of successfully producing offspring<sup>11</sup>. It should be noted that testicular degeneration and atrophy do not occur in all male dogs with canine brucellosis. Infected males may remain sexually active and participate in successful mating although bacteremic and, in some cases, having been infected with Brucella canis for more than a year<sup>54</sup>. This has obvious implications with respect to transmission of canine brucellosis. Occasionally, distention of the scrotum will be observed, which results from an accumulation of fibrinopurulent exudate in the cavity of the tunica vaginalis<sup>11</sup>.



Although the diagnosis of canine brucellosis cannot be established by observation of physical symptoms alone<sup>12</sup>, two clinical features in both sexes are important. First, Brucella canis in the dog causes an afebrile illness<sup>11,12,26,66</sup>. Second, almost all infected dogs will develop enlarged, firm lymph nodes<sup>11,12,26,40,66</sup>, either bilaterally or unilaterally<sup>11,12</sup>. Dogs infected orally typically have enlarged retropharyngeal lymph nodes, whereas those infected by the vaginal route usually have more pronounced enlargement of the superficial inguinal and external iliac nodes<sup>12</sup>. Lymph nodes are palpable approximately two weeks after inoculation with the Brucella canis organism<sup>12</sup>. Additional nonspecific clinical features reported by owners of infected dogs include dry lusterless coats, loss of vigor, tendency to fatigue, and a lack of interest during field trials<sup>11</sup>.

Aborted fetuses may be alive or dead at the time of their expulsion. Most aborted pups will die within 1-3 days<sup>11,12,40</sup>. In some instances, however, pups that presumably were infected in utero survived and developed enlarged lymph nodes as the only clinical sign of illness<sup>12</sup>. Dead fetuses may be in varying stages of decomposition at the time of abortion, or they may appear normal<sup>11</sup>. Some litters, aborted close to term, were reported by owners to have both living and dead pups<sup>11</sup>. In these cases, some pups survived, and others died after two or three days.

#### Pathologic Findings

In a study conducted by Gleiser et al.<sup>33</sup>, the principal pathologic lesions observed in four male and four nongravid female dogs that had

become spontaneously infected with Brucella canis were found in the lymphoid tissues, the principal and accessory male sex organs, and the renal glomeruli. The main abnormalities grossly visible were a lymphadenopathy in five of the eight dogs, and splenic infarcts in four of the animals. These authors concluded that the primary target organs in Brucella canis infections are the principal and accessory sex organs in males. The lesions in the lymphoid tissues and renal glomeruli were considered to be secondary changes (i.e., reflections of and caused by the host's immune response). Microscopically, Gleiser et al.<sup>33</sup> observed the following:

1. Inflammatory lesions of the prostate (4/4 dogs), epididymides (2/4 dogs), and testes (2/4 dogs). The lesions were characterized by a predominantly lymphocytic cellular infiltrate, with some neutrophils, fibroblasts, and plasma cells. Degeneration of testicular germinal epithelium was variable in intensity and distribution. Some atrophic tubules were observed.
2. An increase in the cellularity of all examined lymph nodes was observed in seven (three males, four females) of the eight dogs. The predominant cell type accounting for the increased cellularity was the lymphoblast. Lymphoblasts were especially numerous around the follicles in the cortex of the nodes. Also, large numbers of plasma cells were observed in the medullary cords of four of the dogs.
3. A hyaline thickening of the basement membrane of the glomerular capillaries was seen in seven (four male, three female) of the eight dogs. The deposition of hyaline into the capillary walls

of the glomerular tufts was generalized throughout the renal glomeruli. There was no significant cellular infiltration or proliferation of glomerular cellular elements.

4. Several seemingly incidental microscopic abnormalities were observed, including focal encephalitis in three of the eight dogs, and granulomas in a lymph node of one dog, in the livers of three dogs, and in the lungs of three dogs. Although granulomatous lesions are associated with Brucella infections in other species of animals, it was considered possible by the authors that the granulomas observed in the liver and lungs of these dogs were due primarily to parasitic infections which had been observed to be present in these animals.

It should be noted that Gleiser et al.<sup>33</sup> observed no genital lesions (grossly or microscopically) in any of the four female dogs. The lack of uterine lesions was considered to be a reflection of their nongravid state.

In their studies of the pathology of Brucella canis in spontaneously infected dogs, Morisset and Spink<sup>66</sup> reported extensive hyperplasia of lymphoid tissues, involving even Peyer's patches. Predominant cell types were lymphocytes, plasma cells, and macrophages. Granulomatous lesions of lymphoid tissues were found to be common, while suppurative lesions were uncommon. These authors found the primary pathologic processes of the testicles to be that of degeneration, fibrosis, and atrophy, rather than inflammation. An additional finding in the female was the presence of reticular cell nodules in the endometrium. Although not specified, these females were presumably gravid or recently postpartum,

in view of the fact that this was a study of spontaneous brucellosis in a beagle breeding kennel.

Carmichael and Kenney<sup>12</sup> reported that the pathologic lesions of canine brucellosis were a reflection of the principal involvement of the reticuloendothelial tissues and the vessels of the target organs of gonadal steroids. Generally, the cell types involved were lymphocytes, plasma cells, and reticular cells. The basic tissue reactions were reticular cell hyperplasia of lymphoid organs and granuloma formation elsewhere. Gross lesions in both sexes were limited primarily to generalized lymphadenopathy and splenomegaly. In addition, in the postpubertal male, epididymitis and scrotal dermatitis usually occurred, while in the nonpregnant postpubertal female there was occasionally slight vulvitis. Microscopically, the lymphoid tissues of a dog with canine brucellosis could always be found to be affected. Increased mitotic activity and occasional neutrophils were seen in the core areas of germinal centers. Focal or diffuse accumulations of reticular cells and generalized hyperplasia of lymphocytes were found in the cortical areas of lymph nodes and the white pulp of the spleen. The reticular cells often were prominent in the sinusoids of the corticomedullary junction. In these instances, reticular cells were usually prominent in medullary sinusoids also. Medullary cores were often enlarged, with the predominant cell types being plasma cells. Microscopic lesions in non-lymphoid tissues and organs were usually granulomatous changes involving lymphocytes, plasmacytes, and reticular cells in different combinations. In the lungs, granulomas were usually associated with alveolar ducts. In the liver, granulomas arose in the sinusoids and were focally

distributed. Granulomatous lesions involving lymphocytes were very often found in the gallbladder. A postmortem microscopic finding considered by the authors to be unique for a Brucella infection was chronic meningitis and nonsuppurative encephalitis. Carmichael and Kenney<sup>12</sup> also noted that although orchitis does occur in male dogs infected with Brucella canis, it was not a consistent feature in the animals included in their studies. The authors did note a degeneration of the seminiferous epithelium, which resulted in decreased spermatogenesis. Rather than a true inflammatory process of the testicles, Carmichael and Kenney<sup>12</sup> found more frequently both acute and chronic necrotizing vasculitis. Generally, these types of vascular changes were identified in the target organs of the gonadal steroids - testes, prostate gland, scrotum, sheath, and vulva. Vascular changes were not seen in the uterus. Instead, the authors often found a chronic to subacute endometritis, with or without glandular hyperplasia. Also seen in the uterus was a change that may be unique to canine brucellosis - reticular cell nodules.

In a previous study, Carmichael and Kenney<sup>11</sup> reported the post-mortem findings for dogs experimentally infected with Brucella canis. In their report was a description of the pathological findings associated with a group of bitches which had recently aborted. The uterine cavity of these bitches contained moderate amounts of odorless exudate that varied in color from brownish yellow to greenish brown. The consistency of the exudate ranged from slightly viscous and slimy to tenacious and mucoid in character. Microscopically, there was marked hypertrophy of the glandular epithelium, with focal infiltration of the

lamina propria by lymphocytes, as well as fewer numbers of plasma cells and neutrophils. The myometrium was infiltrated with lymphocytes, and there were occasional small granulomas which were infiltrated by neutrophils. In the uterine lumens were the necrotic remains of portions of the fetal placenta. The basic placental histologic lesion was focal coagulation necrosis of chorionic villi. Also noted in this study were common fetal lesions, which included bronchopneumonia, myocarditis, generalized focal renal hemorrhage with lymphocytic and reticular cell infiltration of the interstitium and perivascular tissue of the pelvis, lymphadenitis, and hepatitis. Often there were areas of focal infiltration of lymphoid cells and fewer neutrophils around sublobular veins of the liver and microfoci of necrosis in portal areas.

In a study of 18 male dogs all naturally infected with Brucella canis, Moore and Kakuk<sup>65</sup> made the following histologic observations:

1. The lymph nodes of all dogs had lesions, consisting primarily of diffuse lymphocytic hyperplasia with infiltration of lymphocytes into the perinodal structures and subcapsular space. The sinusoids were filled with macrophages and plasma cells in advanced lesions.
2. Pathologic changes in the spleen, found in 8 of 15 dogs examined, were histologically similar to changes noted in the lymph nodes.
3. Involvement of the genital system was the histopathologic condition most consistently found. Of 15 dogs examined, all except one had pathologic changes in the prostate gland, epididymides, and at least one testicle. Prostatic involvement was

typified by a generalized lymphocytic infiltration, with extension into, and destruction of, adjacent glandular parenchyma. Fibroblastic elements were commonly seen in areas where there was marked loss of glandular tissue. Of the 14 dogs with testicular abnormalities, nine had lesions in both testicles. Degeneration of seminiferous tubules, which was sometimes the only abnormality observed, ranged from slight to extensive involvement. Extensive loss of seminiferous tubules was followed by replacement with fibrotic tissue interspersed with lymphocytes. By this process, a complete loss of spermatogenic capability could occur in one testicle, while the other testicle retained its spermatogenic ability. The epididymides had accumulations of lymphocytes in the interstitial cell layers ranging from a few cells to large masses of cells; however, little obliteration or stricture of the tubules occurred. Inflammatory cells (lymphocytes, neutrophils, macrophages) were observed in the glandular lumens of both the epididymides and the ductus deferens.

4. Moore and Kakuk<sup>65</sup> noted increased hyalinization of glomeruli, which is consistent with that reported by Gleiser et al.<sup>33</sup>. However, inconsistent with Gleiser et al.<sup>33</sup> was the report by Moore and Kakuk<sup>65</sup> of significant cellular infiltrates in portions of the urinary system. Thus, the latter authors reported a submucosal lymphocytic infiltration in the ureters and bladders of 4 of 15 dogs, a submucosal lymphocytic infiltration of

the renal pelves in all 15 dogs, and seven dogs which showed swollen glomeruli surrounded by lymphocytes.

5. Similar to what was found by Carmichael and Kenney<sup>12</sup>, Moore and Kakuk<sup>65</sup> observed focal lymphocytic accumulations in the lungs of several dogs.
6. In the liver of 2 of 15 dogs, very small necrotic foci were occasionally observed.

In a study by George et al.<sup>32</sup>, the effects on seminal morphology were observed in a group of male dogs experimentally inoculated with Brucella canis. Abnormalities in spermatozoa were not observed until five weeks postinfection, and maximum changes were observed at eight weeks postinfection. Immaturity of the spermatozoa was the first defect noted. Immature sperm were characterized by the retention of perinuclear sheaths, deformed acrosomes, swollen midpieces, and retained protoplasmic droplets. Changes that subsequently occurred included bent tails, head-tail detachment, and head-to-head agglutination of spermatozoa. Clumps of inflammatory cells were observed in the semen from dogs infected between 8 and 35 weeks. The clumps consisted of neutrophils, macrophages and adherent spermatozoa, and phagocytized sperm. Semen from dogs recovered from bacteremia (postinfection weeks 60-100) had few inflammatory cells. Seminal morphology of dogs with unilateral testicular atrophy was similar to other infected, recovered dogs' semen unless the normal-sized testis was removed. Complete aspermia was observed in hemicastrated, recovered dogs that had an atrophic testis remaining. Despite prolonged bacteremia and consistently high serum



titers, the seminal fluid of infected dogs never developed detectable Brucella canis agglutinin titers.

#### "Atypical" Clinical Features In The Dog

To designate a number of clinical manifestations of any disease as "atypical" is obviously an artificial and subjective system of classification. It may very well reflect only the fact that a particular disease has not been studied thoroughly enough so that all facets of that disease are understood. However, such a method of classification may be useful, in that it illustrates aspects of the disease that have been historically emphasized, and other aspects of the disease that perhaps require increased future consideration. Thus, if "typical" symptoms of canine brucellosis are limited to those reproductive problems classically associated with this disease, then "atypical" clinical features of canine brucellosis include the following:

1. Discospondylitis
2. Uveitis
3. Encephalitis

In a study by Hurov et al.<sup>48</sup>, the authors noted that discospondylitis is a specific osteomyelitic disease of the spine caused primarily by various infectious organisms. Their study was retrospective in nature, involving 27 cases of canine discospondylitis which had been diagnosed radiographically at the Texas A & M small animal clinic during the period 1970-1977. A preponderance of male dogs were affected (20/27), and there was a marked prevalence of the condition in large breeds of

dogs (29.6% of the affected animals were great Danes; this breed accounted for only 1.4% of the canines admitted to the clinic during this time period). Discospondylitis was quite common in relatively young dogs. Fourteen were four years of age or less, and nine were less than two years old. Pain, inappetance, recurring fever, and neurologic deficits were the primary clinical signs noted. In serologic tests performed on 14 of the dogs, four were positive for Brucella canis by the slide agglutination test. Confirmation was obtained by use of the 2-mercaptoethanol tube agglutination test, for which a titer of 1:200 or greater was regarded as positive. Culture of blood yielded positive results for Brucella canis in one of ten cases. Biopsy of the vertebral lesion and subsequent bacterial culture also yielded positive results for Brucella canis in one of ten animals. Readily accepted reasons for infectious organisms localizing at the specific site of the intervertebral disk have not become well established. Because hematogenous origin is the most likely source of bacteria, it was recommended that venous channels between the spine and the visceral circulation be considered in more detail in subsequent studies.

Henderson et al.<sup>39</sup> reported on the occurrence of discospondylitis in three dogs (one poodle-type dog and two German shepherds, all males). Clinical signs upon presentation included spinal pain and posterior muscle weakness. Spinal radiography revealed radiolucent areas and sclerosis consistent with discospondylitis. Brucella canis was isolated from discospondylitic lesions of one or more intervertebral spaces from each of the three dogs. Serological determination of Brucella canis titers was positive at 1:200 (highest dilution measured) for all three dogs.

No attempt was made to culture the organism from the blood, because brucellosis was not suspected initially, and the dogs were no longer available or had been started on tetracycline therapy by the time Brucella canis was identified. Brucella canis was isolated from the epididymides and testicles of two dogs (testicles of the third dog were not cultured). Two dogs recovered after spinal decompression, vertebral curettage, and oral tetracycline therapy. The third dog was euthanized at the owner's request.

In a study by Hubbert et al.<sup>47</sup>, 158 pet dogs were examined and categorized as either "Healthy", "With reproductive disorders", or "With nonreproductive disorders". Of the 39 dogs in the latter category, 12 were diagnosed as having discospondylitic lesions. Of these 12, six were positive serologically for Brucella canis (positive slide agglutination test, positive 2-mercaptoethanol tube agglutination test - titer 1:200 or more).

The possible causal link between Brucella canis and canine discospondylitis is not without precedent. The predilection of organisms of the Brucella group for osseous tissues of swine was noted by Feldman and Olson<sup>22</sup> in 1933. These investigators obtained 24 swine with spondylitic lesions from various abattoirs. Based on total slaughter numbers during the period in which some of the 24 cases of spondylitis were noted, the authors estimated the prevalence of spondylitis (presumably due to all causes) in swine to be approximately 1 in 6,000. The authors noted no age, breed, or sex predisposition. The spondylitic lesions were encapsulated, abscess-like structures occupying an irregular cavitation in the body of the vertebrae, usually in the lumbar and sacral regions. By

direct culture and from the tissues of the inoculated animals, organisms belonging to the genus Brucella (probably Brucella suis) were isolated from 10 of the 24 cases studied.

Chronic osteomyelitis, especially with involvement of the vertebrae, has been noted among farmers, meat packers, and those who drink unpasteurized milk<sup>1</sup>. Incriminated species of Brucella include abortus, melitensis, and suis. The osteomyelitic lesions are usually accompanied by the classical symptoms of Recurrent Fever (i.e., fever, weight loss, et cetera).

A second "atypical" feature of canine brucellosis, that of ocular involvement, has been noted by Saegusa et al.<sup>81</sup>. In studies performed on three experimentally inoculated beagles, these authors noted recurring corneal opacification in two of the animals. One beagle was bilaterally affected, with more severe changes noted in the right eye. Four occurrences of such ocular involvement were noted over a 382 day period (total time from experimental intravenous inoculation until euthanasia and necropsy). The ocular lesions were first noted on day 238 postinoculation, and the duration of individual episodes of corneal opacification ranged from 1-5 weeks. The other affected beagle had involvement only of the left eye. Corneal opacification was first detected on day 217 postinoculation, and there were three occurrences over a 385 day period (total time from inoculation until euthanasia and necropsy). The duration of individual episodes of ocular involvement ranged from 3-10 weeks. The third episode of the second dog involved only hyphema of the left eye without corneal opacification. The Brucella canis organism was recovered from the blood of the second dog during the course

of the illness, and from the aqueous fluid of the eyes of both beagles. Agglutinin serum titers to Brucella canis were noted in both dogs (up to 1:640 in the first dog; up to 1:1280 in the second dog). Agglutinin titers of the aqueous fluid for both dogs were at times equal to or greater than those of the serum sampled at corresponding times (the second dog did not develop a significant titer in the aqueous fluid of the right eye). Histologic changes noted in both dogs included nongranulomatous iridocyclitis and exudative retinitis. In the iris, there was a diffuse infiltration of plasma cells and circumscribed lymphoid nodules near the encircling artery. In the ciliary body, there was also a diffuse infiltration of plasma cells plus congestion and hemorrhage. The corneal endothelial cells were vacuolated and detached from the Descemet's membrane, where moderate infiltration of plasma cells and neutrophils was seen. Serous exudate and some leukocytes were seen in the anterior chamber as well as in the vitreous body. There was diffuse infiltration of plasma cells between the inner plexiform layer and the nerve fiber layer of the retina. Some serous exudate and a few lymphocytes were seen between the choroid and the detached retina. Sometimes an infiltration of plasma cells and lymphocytes was seen in the choroid layer, and some lymphocytes were accumulated around the anterior ciliary vein in the scleral border.

Ocular involvement was also noted by Riecke and Rhoades<sup>78</sup>, who reported on a 2 1/2-year-old female German shepherd which was presented to a veterinarian because of anorexia and listlessness. No diagnosis was made initially. One week later, the dog was returned with traumatic hyphema, and the eye was treated symptomatically for two months. At

that time, the cornea was completely opaque. Topical and systemic treatment were continued, but the eye did not respond. One month later the eye was atrophied, white, and nonfunctional. A sample of aqueous humor was obtained by paracentesis, and a pure culture of Brucella canis was isolated. Subsequent testing of the dog's serum for Brucella canis agglutinins resulted in a titer of 1:800. No attempt was made to culture the blood, and it was not possible to determine how the dog contracted the disease. Ocular involvement due to Brucella species, like discospondylitis, is not unprecedented. Riecke and Rhoades<sup>78</sup> noted that ocular problems in humans have been associated with infections by other Brucella species, as previously reported by Opperman et al.<sup>69</sup>.

The third "atypical" feature of Brucella canis in dogs is that of central nervous system involvement. Carmichael and Kenney<sup>12</sup> reported that a lesion which is considered unique for a Brucella infection is chronic meningitis and nonsuppurative encephalitis. Harris et al.<sup>37</sup> investigated a natural case of Brucella canis infection in a research canine colony. The animal involved, an adult female beagle, was observed in grand mal convulsions. Convulsive episodes were characterized by strong paddling of the forelegs and mild hindlimb paddling, clenched teeth, and profuse salivation. Despite intensive therapy for four days, the animal's condition deteriorated, and it was euthanized to prevent further suffering. At necropsy the spleen, blood, and spinal fluid were cultured, and Brucella canis was isolated. There were no gross lesions in the brain. However, microscopically there was widespread endothelial swelling and proliferation with associated perivascular cuffing by mononuclear cells. To further study the central nervous system effects

produced by Brucella canis, Harris et al.<sup>37</sup> experimentally infected four other beagles with the isolate obtained from the naturally infected dog. Although none of the four dogs displayed clinical signs, Brucella canis was cultured from the brain of one of the dogs at necropsy. Microscopic examination of this brain revealed a diffuse meningoencephalitis characterized by a subacute inflammatory reaction around several vessels in the cerebrum and medulla. Furthermore, these authors quoted a personal communication with L. E. Carmichael, in which he stated that a high percentage of Brucella canis infected dogs display a histologic encephalitis without corresponding clinical signs. Thus, Harris et al.<sup>37</sup> concluded that while Brucella-associated encephalitis is usually mild and thus subclinical, an atypically virulent strain or susceptible dog cannot be dismissed from consideration in naturally occurring cases of central nervous system involvement.

#### Transmission

Carmichael and Kenney<sup>11</sup> showed that experimental transmission of Brucella canis can be accomplished by various routes of inoculation.

Using strain RM-666, these investigators observed and reported the following:

<u>ROUTE</u>	<u>NO. INFECTED/NO. EXPOSED</u>	<u>INCUBATION PERIOD*</u>
Intravenous	10/10	4 to 9 days
Subcutaneous	6/6	7 to 14 days
Oral	11/12	7 to 21 days
Intravaginal	3/3	7 days
Contact**		
vaginal discharge	4/4	7 to 21 days
infected male	1/3	14 days

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\*Time between exposure and development of bacteremia as determined by cultures of blood.

\*\*Bitch with vaginal discharge placed in isolation unit with susceptible dogs; infected male with epididymitis placed in unit with three susceptible females.

These authors also noted that natural transmission of Brucella canis probably occurs most frequently through direct contact with infective vaginal discharges or aborted fetal and placental tissues. Aborted placental tissues and vaginal discharge fluids may contain up to  $10^{10}$  organisms per ml.<sup>8</sup>; the oral-infectious dose for dogs is approximately two million colony-forming units<sup>8</sup>. Transmission by this route may continue for 4-6 weeks after an abortion<sup>8</sup>. In one experimental setting, Carmichael and Kenney<sup>11</sup> placed four disease-free dogs in an isolation unit with a bitch that had aborted two days previously. All four dogs became infected within three weeks. In another experiment, Carmichael



and Kenney<sup>11</sup> allowed three experimentally infected dogs (two males, one female) to live with two uninfected female littermates in an isolation unit. All dogs were six months old at the onset of the experiment. The uninoculated dogs did not become infected during a ten month period, suggesting that spread via urine, saliva or feces does not readily occur. However, after eleven months, the two uninoculated females were found to be infected. Because both bitches were observed in heat three weeks prior to onset of bacteremia, it seems likely that infection had occurred during breeding. Thus, the venereal spread of Brucella canis also appears to be an important factor in the natural transmission of this disease. Brucella organisms can usually be isolated from the epididymides and prostate gland (as well as lymphatic tissues) of infected males<sup>8,11</sup>. These tissues serve as sites of persistent infection, and intermittent shedding of organisms in the semen has been observed for periods up to 60 weeks<sup>8</sup>. Brucella organisms have been cultured from the prostate and epididymal tissues of some dogs for periods exceeding two months after cessation of the bacteremia<sup>8</sup>. Although the number of organisms in the semen is high only in the initial one to two months following infection, venereal transmission probably occurs readily even though the number of organisms is low<sup>75</sup>. Conclusive data concerning a minimal venereal-infectious dose are lacking<sup>75</sup>. Venereal transmission also occurs between uninfected males and infected females in heat<sup>75</sup>.

Although contact with urine from infected dogs does not appear to be a major mode of natural transmission, such transmission is possible. This fact should be considered when planning prevention and

control activities, since the Brucella canis organism has been recovered from the urine of infected dogs<sup>11,65,83,84</sup>. Out of 11 dogs experimentally infected by Carmichael and Kenney<sup>11</sup> (gravid and aborting females excluded - sexes otherwise not stated), one was found to have Brucella canis organisms in the urine (cultured from the urine 3-4 months post-inoculation). Moore and Kakuk<sup>65</sup> made the following observations in a study of natural infection in a beagle colony:

18 male dogs - agglutinating titer 1:200 or more

1. SUSPECT GROUP - never had bacteremia (six dogs).
  - Brucella canis not cultured from bladder urine of any of the six.
2. INFECTED GROUP - bacteremia present (12 dogs).
  - a. long-term bacteremia - at least 103 days (five dogs).
    - Brucella canis cultured from bladder urine of four of these dogs.
  - b. short-term bacteremia - average bacteremic period: 77 days (seven dogs).
    - Brucella canis cultured from bladder urine of one of these dogs.

Serikawa et al.<sup>84</sup> noted a difference between males and females with respect to the occurrence of Brucella canis organisms in the urine of spontaneously infected animals. By direct culture, these authors isolated Brucella canis from bladder urine in 14 out of 16 males (87.5%), and only five out of 17 females (29.4%). In addition, the

number of viable Brucella canis organisms in bladder urine was greater in males than in females. Serikawa et al.<sup>84</sup> also noted a slightly higher frequency (though not statistically significant) of rates of isolating Brucella canis from the renal medulla in males than in females. No sex difference was noted in the renal cortex. Also, isolation rates from urine in males were almost the same as rates of isolation from the prostate. All of these observations may be accounted for by the close proximity of the prostate and ductus deferens to the bladder in the male. These tissues, in which the Brucella canis organism persists in males, may periodically disseminate organisms into the urinary tract, which either ascend to the bladder, or are excreted in urine. Thus, in males, Brucella canis organisms in the urine may originate from the prostate, ductus deferens and kidney, while in females, the organisms originate only from the kidney. In a later study, Serikawa and Muraguchi<sup>83</sup> experimentally infected five male beagles orally. Brucella canis organisms were detected in blood samples two to six weeks after inoculation, and in urine samples one to four weeks after the onset of bacteremia. Urinary excretion continued for at least three months, with the levels of organisms fluctuating. The highest concentration,  $10^6$  organisms per ml, appeared sufficient for transmission. Five 4-week old mongrel puppies were allowed to cohabit with the infected males from the time when the latter were inoculated with Brucella canis. One of the puppies became infected with Brucella canis, as demonstrated in the tenth week of cohabitation by serological testing (titer 1:320). Based on records of when and in what quantities the Brucella canis organism was found in the urine of the five male dogs, it seems that

the most probable mode of transmission to the puppy may have been an intake of contaminated urine. It was suggested by the authors that the reason why only one of five puppies became infected was that the chance of contacting infective urine was somewhat rare. If the puppies had been housed in a facility small enough to provide frequent chances of direct contact with infective urine, all of the puppies may have become infected. Serikawa and Muraguchi<sup>83</sup> also were able to reasonably discount other methods of transmission (i.e., venereal, congenital or via milk, salivary, fecal and vector).

Another suggested mode of transmission of Brucella canis is through the milk of infected bitches. A number of investigators<sup>8,61,75</sup> have shown that the mammary secretions of infected females contain abundant bacteria, thus providing an additional source of environmental contamination. Although infective milk probably serves as a means of transmission to offspring and other adults, conclusive data on this point are lacking.

The congenital (transplacental) transmission of the Brucella canis organism is also known to occur<sup>18,52</sup>. Although in most instances of Brucella abortion the fetuses are stillborn, a modest proportion of pups survive. Surviving pups, even if they appear healthy, may have generalized lymphadenopathy and Brucella canis bacteremia. Because few dogs infected this way survive, congenital transmission is probably of little importance.

Because of the prolonged bacteremia, blood transfusions taken from asymptomatic carriers or the use of contaminated needles or syringes could also be possible routes of transmission<sup>75</sup>.

Case-associated fomites and caretakers, and mechanical spread of aborted material by rodents and insects have also been incriminated in the spread of Brucella canis<sup>54</sup>.

Aerosol transmission of Brucella canis in the close confines of a kennel setting may possibly occur<sup>18</sup>. This seems plausible in view of the fact that inhalation of infective aerosols has been incriminated as the mode of transmission of Brucella suis and Brucella abortus to abattoir workers<sup>79</sup>. Conclusive experimental data on the aerosol transmission of Brucella canis are lacking.

A final, and probably minor, mode of transmission is via the saliva of infected animals. Brucella canis organisms have been isolated from the salivary glands of infected dogs by some investigators<sup>83,84</sup>. Thus, transmission of canine brucellosis may conceivably occur through direct contact with salivary secretions, or by an infected animal biting a susceptible animal. The latter mode of transmission was believed to have occurred in the case of a hog farmer who was accidentally bitten by his pet dog, and who subsequently developed an illness, during which Brucella suis was isolated<sup>79</sup>.

#### Predisposing Factors

##### 1. Breed

Because this disease was first recognized and studied in large breeding colonies of beagles used for field-trial and commercial purposes, it was initially suspected that the beagle-breed was especially susceptible to the agent of canine abortion<sup>8</sup>. The first descriptive report of the nature of the disease appeared in a

distinguished journal of beaglers, "Hounds and Hunting"<sup>9</sup>, in 1967. The author of this article, L.E. Carmichael, one of the original investigators of canine brucellosis, stated in another publication co-authored with R.M. Kenney in 1970<sup>12</sup>, "the disease was recognized initially in beagles, and it still appears to be most prevalent in this breed .... studies in the laboratory, however, have not revealed any particular breed susceptibility". Obviously, the question of breed predisposition was initially a point of confusion. However, it is now generally accepted that all breeds, mixed and pure, are equally susceptible to the Brucella canis organism. In a 1976 publication<sup>8</sup>, L.E. Carmichael states "there is no particular susceptibility of this breed (beagle) .... many additional breeds, as well as mixed-breed dogs have been found infected". Hill et al.<sup>40</sup> reported on an epidemic of canine brucellosis in a dog breeding colony. Abortions occurred in beagles, pointers, greyhounds, and crossbred animals. The distribution of abortions by breeds approximated the distribution of the breeds of the affected females, suggesting that various breeds do not differ in their susceptibility to infection. In a study by Hubbert et al.<sup>47</sup>, 296 stray and pet dogs were serologically tested for Brucella canis agglutinins. The results of these investigators showed a lack of correlation between seropositive reaction and breed.

## 2. Sex

Males and females appear equally susceptible to the agent of canine brucellosis. Also in the survey of stray and pet dogs by Hubbert et al.<sup>47</sup>, no correlation was detected between seropositive agglutination results and sex of the animals involved. In a study

of 650 pet and stray dogs by G.E. Lewis<sup>53</sup>, one in five dogs demonstrated a complete agglutination titer at a dilution of 1:100 or greater. Of these, 51% were females and 49% were males - the difference was not significant. Serikawa et al.<sup>84</sup> serologically tested 1,186 stray dogs from the Gifu and Shiga areas of Japan. They found no statistically significant difference between the number of seropositive males and the number of seropositive females.

### 3. Age

Age does not appear to be a predisposing factor, other than in the decreased resistance/increased susceptibility of the very young puppy and older, debilitated animal to infectious agents in general. Flores-Castro and Carmichael<sup>23</sup> noted that puppies do not appear to possess the relative resistance seen in calves, kids, and young human beings to Brucella species. However, the sexually mature dog would have higher potential of contracting canine brucellosis through the venereal mode of transmission. In their study of various purebred and cross-bred dogs in a canine production colony, Hill et al.<sup>40</sup> noted abortions in females aging from 13-60 months. These investigators could detect no differences in susceptibility by age within this group. In a 1977 publication<sup>3</sup>, C.L. Barton noted that "there is apparently no age .... predilection, although obviously the sexually mature intact canine is favored".

### 4. Stray versus pet

Probably the most significant predisposing factor is whether a dog is owned and confined, or not owned with uncontrolled movement. Generally, a higher prevalence rate of Brucella canis exposure is

observed in stray dogs, relative to pet dogs. This difference is presumed to be related to an increased opportunity of the stray group for exposure through multiple breedings and other contacts with infected dogs, as compared with the more restricted movement and decreased opportunity for exposure of the nonstrays. Numerous surveillance studies in the United States have detected significantly different prevalence values for nonstrays and strays, with average figures approximating one and eight percent, respectively<sup>5,6,27,29,57</sup>. Interestingly, a study by Saegusa et al.<sup>80</sup> of 945 stray and pet dogs from the Tokyo area revealed no significant difference in positivity rates between stray and nonstray dogs. Although the authors noted that this observation differed from previous results observed in the United States, they did not attempt to explain the inconsistency.

#### 5. Urban versus rural

Wooley et al.<sup>97</sup> conducted a serological survey of 100 urban stray dogs and 100 rural stray dogs in the area of Atlanta, Georgia. Three of the rural strays were positive by tube agglutination test at a titer of at least 1:100, while nine of the urban strays were positive at this titer. This difference between the positivity rates of the two populations was not statistically significant. These results were supported by observations made by Thiermann<sup>91</sup> in a study of 499 urban strays and 123 suburban strays in the area of Detroit, Michigan. By tube agglutination test, 8.6% of the urban strays and 5.7% of the suburban strays were found to have titers of 1:200 or greater to the Brucella canis organism. Although this investigator did not perform a statistical analysis of his results, a chi-square test for goodness of fit



reveals that there is no significant difference (.05 level) between the two percentages noted above. It would appear, in view of these studies, that urban and rural populations of stray dogs are affected to essentially the same extent by the agent of canine brucellosis.

#### 6. Geographical location

C.L. Barton<sup>3</sup>, in a 1977 review, noted that the prevalence of Brucella canis infection varies greatly from location to location in the United States, but would appear to be greatest in the southern portion of the country. Carmichael and Kenney<sup>11</sup> stated in an earlier publication that, based on reports and kennel testing procedures, "it (canine brucellosis) seems to be widespread throughout the United States". R.V. Pollock<sup>75</sup> reported in 1979 the results of a study performed by the Baker Institute, in which it was estimated that 0.5 to 1.5% of the dogs in the northeast United States were infected with the Brucella canis organism. Lovejoy et al.<sup>57</sup> in a 1976 publication, related the results of a serologic study of 2,000 dogs from the southwest United States, in which a positivity rate of one percent was determined. These generalizations should be interpreted with caution, for observations such as these depend not only on the distribution of the Brucella canis organism, but also on such variables as availability of facilities for testing animals, the interest and participation of animal owners, and degree of public education concerning this problem. Results of specific serologic surveys from various regions of the United States (as well as from other countries) will be considered in detail in the following section.

## 7. Season

Little information is available concerning the effect of season on the occurrence of canine brucellosis in dog populations. In a study of 1,186 stray dogs from the Gifu and Shiga areas of Japan, Serikawa et al.<sup>84</sup> noted that monthly detection rates of infected dogs fluctuated from 0.8 to 5.5%. However, no clear seasonal influence was observed by these investigators.

## Prevalence Studies in Various Geographic Regions

The reported seroprevalence of canine brucellosis in dogs varies widely, as shown in Table 1. There exists an obvious diversity of reported prevalence values, which reflects not only probable differences in the distribution of the Brucella canis organism, but also various shortcomings of serological procedures. For example, as noted in Table 1, there is not even a consensus among investigators as to what magnitude of serological titer they should consider significant. As Pollock<sup>75</sup> notes, "the range reflects differences in locale, technique and interpretation and emphasizes the fallibility of serologic techniques in this disease". Methods of diagnosis of canine brucellosis, including a comparison of serologic methods, will be detailed in the following section.

## Diagnosis

Canine brucellosis is an insidious, often asymptomatic disease, and therefore cannot be diagnosed by clinical signs alone, especially in prepubertal and nonpregnant dogs. Therefore, the clinician must

utilize not only physical findings, but also the history of the animal, as well as serologic and bacteriologic diagnostic methods. Thus Brucella canis infection should be included in a differential diagnosis when the female exhibits or has a history of spontaneous abortions during the last trimester of pregnancy, or when physical changes such as epididymitis or testicular atrophy occur in the otherwise healthy male<sup>63</sup>. These signs, as well as others seen in canine brucellosis (e.g., whelping of stillborn pups, repeated conception failures, lymph node enlargement, etc.) are not pathognomonic; infection with beta hemolytic Streptococcus, Escherichia coli, and Herpesvirus canis, and various endocrine disorders may elicit similar signs<sup>63</sup>.

The primary methods of serological testing include the rapid slide agglutination test (RSAT), tube agglutination test (TAT), 2-mercapto-ethanol tube agglutination test (ME-TAT), and agar gel immunodiffusion test (AGID). Flores-Castro and Carmichael<sup>23</sup> concluded that none of these commonly used procedures is, in itself, adequate to permit a definitive diagnosis in all cases. The diagnosis is simplified when a number of animals in a kennel are infected; however, individual cases pose a variety of problems, especially when complete clinical histories are not available or opportunities for infection have not been discovered. Because serologic methods often are not totally adequate, arriving at a definitive diagnosis requires isolation of Brucella canis (usually from the blood), by bacteriologic methods. However, as Flores-Castro and Carmichael<sup>23</sup> also noted, bacteremia is frequently absent in chronically infected dogs, and laboratory diagnosis (serologic and bacteriologic) often is not possible without repeated samplings.

The RSAT (or plate agglutination test) for Brucella canis was first developed by George and Carmichael<sup>30</sup> at Cornell University in 1973. This test used a killed, stained (brilliant green/crystal violet) whole cell culture of Brucella ovis to detect antibodies to Brucella canis in suspect serum. Advantages of the RSAT included ease of performance, simplicity in interpretation, and accuracy in detecting infection<sup>13</sup>. Brucella ovis was utilized because it is antigenically almost identical to Brucella canis, Brucella canis tended to form gelatinous suspensions that could not be overcome by laboratory treatment, and because nonspecific agglutination commonly occurred with the canine Brucella<sup>13</sup>. George and Carmichael<sup>30</sup> evaluated the RSAT by comparing its results with the results of tube agglutination tests on eleven experimentally infected dogs. They found that both tests correlated with bacteremia for samples obtained three or more weeks after the onset of bacteremia, while neither test correlated with bacteremia for samples obtained less than three weeks after Brucella canis was first isolated from blood cultures. Also, George and Carmichael<sup>30</sup> found complete agreement between plate and tube agglutination test results on 147 serum samples from dogs in a normal field situation.

In 1974, House and Badakhsh<sup>45</sup> reported on a study they performed, in which they tested 2,367 canine samples (randomly selected from the 1972 survey for Venezuelan equine encephalitis) using the slide test reported by George and Carmichael<sup>30</sup>. House and Badakhsh<sup>45</sup> compared their results with those obtained by two other researchers who had tested the same serum samples using a TAT. House and Badakhsh<sup>45</sup>

calculated a percentage of agreement (between RSAT and TAT) or "correlation" of 99.1%. They also calculated a percentage of disagreement of less than one percent. It should be noted that House was associated with the Research Division of Pitman-Moore, Inc., and another article by House<sup>44</sup> was published in 1974. This publication emphasized two Pitman-Moore products - one for culturing the Brucella canis organism (Bactassay<sup>R</sup>: Pitman-Moore, Inc., Washington Crossing, N.J., 08560), and one for performing a rapid slide agglutination test to detect exposure to the Brucella canis organism (Canine Brucellosis Diagnostic Test Kit: Pitman-Moore, Inc., Washington Crossing, N.J., 08560). As originally described and used by George and Carmichael<sup>30</sup>, the RSAT was semiquantitative. This was accomplished by varying the amount of test serum mixed with the Brucella ovis suspension - with agglutination scored from +1 to +4. Then, specific scores at specific serum dilutions were designated as diagnostic of infection. In this manner, George and Carmichael<sup>30</sup> were able to demonstrate a high sensitivity and specificity for their RSAT. However, the kit form of the RSAT (Pitman-Moore) is qualitative only, with just one dilution of serum tested. The result is a high number of false positive results. These false positive results apparently occur as a result of cross-reaction between antibodies to a number of organisms, including Bordetella bronchiseptica, Pseudomonas sp., and a Moraxella-like organism and the surface antigens of Brucella ovis<sup>75</sup>. Brown et al.<sup>6</sup> found that the RSAT kit (Pitman-Moore) identifies as much as 58% false positive, relative to the TAT. These investigators ignored the reported "99% correlation", and performed their own analysis of the

manufacturer's data. They found that the RSAT kit is very accurate when the results are negative (99.7% sensitivity) but less so when the results are positive (62.5% specificity). In 1978, George and Carmichael<sup>31</sup> reported on the preparation, standardization, and testing of a rose bengal stained plate-test antigen. Their study showed that rose bengal plate-test antigen and crystal violet plate-test antigen gave comparable results in experimental cases of canine brucellosis. With field sera, however, the rose bengal antigen was more sensitive than either the crystal violet antigen or a tube agglutination test antigen. The Pitman-Moore RSAT kit currently uses a rose bengal test antigen<sup>75</sup>, and also provides for the use of 2-mercaptoethanol to decrease nonspecific agglutination reactions<sup>74</sup>. In spite of the large number of false positive results obtained with the RSAT, it is valuable as a screening device, because it is simple to perform, available to practitioners, and is inexpensive. A negative result on a RSAT indicates that the dog is indeed probably free from canine brucellosis. A positive result is not as conclusive, and serum should be forwarded to a diagnostic laboratory for further testing.

The TAT is a widely used diagnostic test having a much greater specificity than the RSAT, resulting in fewer false positive reactions. However, the TAT is much more difficult to perform and takes much longer than the RSAT. The TAT uses an antigen consisting of heat-killed, washed Brucella canis organisms adjusted to a specified optical density<sup>75</sup>. Serial dilutions of test serum are prepared, and incubated at 37°C for 48 hours<sup>42</sup>, at which time the degree of agglutination is judged by clearing of the supernatant. The ME-TAT is similar to the

TAT, except the former provides for the addition of 2-mercaptoethanol (2-ME) to the stock antigen-test serum solution. The purpose of the 2-ME is to increase the specificity of the test by denaturing non-specific cross-reacting 19S agglutinins<sup>75</sup>. In experimental work performed by Flores-Castro and Carmichael<sup>23</sup>, they found that titers obtained by the ME-TAT generally were two-fold lower than those obtained by the TAT, until the 48th week of infection. They also noted that analysis of results obtained by TAT and the ME-TAT revealed that differences between the number of positive reactors detected by each of these methods were not statistically significant. However, there were significant differences between the number of suspicious and negative samples detected by these tests (i.e., more negative samples were registered by the ME-TAT than by the TAT, and just the opposite for suspicious results). Various investigators differ in opinion as to what is considered a significant TAT or ME-TAT titer. However, serologic evidence of infection generally is indicated by TAT titers in excess of 1:200 and ME-TAT titers in excess of 1:100<sup>24</sup>. There is good correlation between TAT titers of 1:200 or greater and definitive proof of infection by recovery of the organism on blood culture<sup>26</sup>. In experimental infections of specific-pathogen-free dogs, Flores-Castro and Carmichael<sup>23</sup> observed bacteremia in 75% of the animals four weeks after experimental inoculation. Antibody responses first were detected shortly after the onset of bacteremia; however, antibody levels that would suggest infection did not occur until about four weeks later. Meaningful ME-TAT titers occurred 1-2 weeks after TAT titers had reached levels of 1:200.

The AGID test has only recently been adapted for use in diagnosing canine brucellosis, and its availability is limited. The test, as described by Pollock<sup>75</sup>, employs six equally spaced peripheral wells cut into an agarose gel surrounding a central well. The peripheral wells are filled with aliquots of known positive, known negative, and test sera. A sodium deoxycholate extract of heat killed Brucella canis is placed in the central well. Antigen diffusing outward meets precipitating antibody diffusing from the peripheral wells. Where an antibody-antigen reaction takes place, a distinctly visible precipitin line forms. A great advantage of the AGID test is that, by observing the shape of resulting precipitin lines, it may be determined whether the reaction involves homologous or heterologous antibodies. The specificity of this test is thus improved over the previous serologic methods of diagnosis. It may be concluded that the single most reliable serologic method is the AGID test<sup>26</sup>. However, when possible, all four serologic tests should be performed on serum samples. Flores-Castro and Carmichael<sup>23</sup> showed that out of 411 sera tested by the RSAT, TAT, ME-TAT, and AGID techniques, reasonable (not infallible) judgements could be made on only about 90% of the samples.

Several other techniques for serologic diagnosis including micro-titer plate agglutination, fluorescent antibody techniques and complement fixation tests have been described<sup>75</sup>. Myers et al.<sup>68</sup> describe a microslide gel-diffusion serologic test for Brucella canis. However, none of these techniques are in general use.

As noted earlier, definitive diagnosis of canine brucellosis is dependent upon the bacteriologic isolation of the causative agent.



Although isolation of Brucella canis is usually made from the blood of affected animals, the organism may also be recovered from lymph nodes, bone marrow, milk, urine, vaginal discharges and semen<sup>8,75</sup>. Because the number of Brucella canis organisms in the blood (or other tissue) may be low, the blood (or other tissue) is inoculated into an enrichment broth (Brucella or tryptose) for five days of incubation at 37°C, and then transferred to Brucella or tryptose agar<sup>75</sup>. Positive identification is made by observation of differentiating characteristics (e.g. colonial morphology, biochemical reactions, etc.). In a study by Serikawa et al.<sup>85</sup> involving 151 stray dogs of both sexes, it was concluded that urine-culture is effective in males for detecting Brucella canis infection, even when blood-culture is negative. However, in females, urine-culture was not as successful, because urine samples were frequently contaminated (probably related to the difficulty of catheterization) and because infected females usually have fewer organisms in the urine than infected males.

As with any disease, problems of interpretation of diagnostic results are encountered. For example, chronically infected males may be negative serologically and bacteriologically, and yet may harbor the Brucella canis organism in the epididymis and prostate<sup>26,98</sup>. Antibiotic therapy for brucellosis or other unrelated infections may lead to false negative serologic and bacteriologic results<sup>75</sup>. After treatment is discontinued bacteremia may recur, and the antibody titer may increase to its former level. If diagnostic tests were performed during the period of treatment, their results may not be representative of the true infection status of the animal. Serum

samples for serology must be free of hemolysis, which is difficult to accomplish, because dog blood hemolyzes readily<sup>8</sup>. However, hemolyzed samples may produce autoagglutination, resulting in false positive reactions<sup>75</sup>. Finally, chronically infected animals may have marginal titers, which presents a dilemma in interpretation<sup>26</sup>.

### Treatment

With respect to an effective therapy regimen, the prognosis for Brucella-infected dogs is grave<sup>75</sup>. While the disease itself is not life-threatening to the dogs and spontaneous recovery may occur, such recovery may take up to three years<sup>75</sup>. During this time the animal serves as a continual source of infection for other dogs and humans.

A number of in vitro studies have been performed in which the susceptibility of Brucella canis to various antibiotics was examined. Hall and Manion<sup>36</sup> tested four strains of Brucella canis, along with 23 other strains of Brucella suis, Brucella abortus, Brucella melitensis, Brucella ovis, and Brucella neotomae. With respect to all of the Brucella species, the tetracycline class of antibiotics was the most effective. Although chlortetracycline was effective, demethylchlortetracycline and tetracycline were found to be even more so. Erythromycin, gentamicin, streptomycin, kanamycin, and rifampin were quite active. The penicillin-cephalosporin group, with the exception of ampicillin, was comparatively ineffective, as were the polypeptides (colistin, polymyxin-B) and the miscellaneous group of chloramphenicol, lincomycin, cycloserine, and sulfadiazine. A previously unreported finding was noted, in that some strains of Brucella canis were

considerably more resistant to streptomycin and the tetracyclines. Terakado et al.<sup>90</sup> tested 90 strains of Brucella canis for antibiotic sensitivity. All strains were highly susceptible to tetracyclines (chlortetracycline, oxytetracycline, tetracycline, etc.), and the aminoglycosides (streptomycin, kanamycin, gentamicin, etc.). The strains were also susceptible to chloramphenicol, spectinomycin, rifampin, and sulfonamide. However, in these 90 isolates, decreased susceptibility was found to cephalosporin antibiotics and nalidixic acid, and almost insensitivity to polypeptide antibiotics (colistin, polymyxin-B, bacitracin) and cycloserine. Also, 24.4% of the 90 strains were found to be multiply resistant to macrolide antibiotics, some of the penicillins, novobiocin and lincomycin. These multiple drug-resistant strains were isolated from stray dogs and breeding beagles in various districts, indicating that the prevalence of such resistant strains was relatively wide-spread in dogs. Because some of these multiply resistant strains undoubtedly arose through the use of therapeutic drugs, it was concluded that the choice of drug for the treatment of Brucella canis infection must be handled carefully.

Many in vivo antibiotic regimens have been tested. Typically, the persistent bacteremia which characterizes this disease will be eliminated only for the duration of treatment. Antibody titers, which tend to parallel the bacteremia, decline several weeks after the organism has disappeared from the blood<sup>11</sup>. Following cessation of the course of treatment, dogs usually again develop bacteremia within one to two months, followed by a rise in antibody titer<sup>11</sup>. Lewis et al.<sup>56</sup> tested the therapeutic value of tetracycline and ampicillin in dogs

experimentally infected with Brucella canis. Four dogs were treated with tetracycline HCl (250 mg.) orally t.i.d. for 21 days, and four other dogs received ampicillin (250 mg.) orally t.i.d. for 21 days. All of the dogs infected with Brucella canis harbored this organism before and after antibiotic therapy. None of the treated dogs were completely cleared of the organism, as confirmed by blood and tissue cultures. Jennings et al.<sup>49</sup> reported on the effect of a two-stage antibiotic regimen for dogs experimentally infected with Brucella canis. In this study, ten adult beagle dogs were inoculated with Brucella canis. When infection was confirmed four weeks later, four of the dogs were treated with ampicillin for three weeks, four were treated with tetracycline for three weeks, and two dogs received no treatment. Fifteen weeks later, all ten dogs were treated for three weeks with streptomycin and tetracycline. At necropsy, 36 weeks after inoculation, seven of the eight twice-treated dogs had Brucella canis-agglutinating titers of less than 1:100 and Brucella canis could not be isolated from selected tissues. The two dogs given the tetracycline-streptomycin treatment, without prior antibiotic treatment, had Brucella canis-agglutinating titers of 1:400 and 1:800 and Brucella canis was isolated from cultures of selected tissues. The authors concluded that the selection of drugs did not seem to be as important as the staging of treatment. Pollock<sup>75</sup> has noted a number of weaknesses in the study by Jennings et al.<sup>49</sup>. First, the number of dogs used in this study (ten) was small. Also, the animals used were experimentally infected for only a short time (four weeks) prior to initiation of therapy. Whether chronically infected dogs

would respond as readily to this regimen is not known. Johnson et al.<sup>50</sup> studied the effect of combined antibiotic therapy on fertility in brood bitches infected with Brucella canis. Six female beagles, with naturally occurring Brucella canis infection were treated as follows: tetracycline t.i.d. (orally) for 14 days, followed immediately by dihydrostreptomycin b.i.d. (IM) for 14 days, followed immediately by trimethoprim-sulfadiazine b.i.d. (orally) for 14 days. Although this sequential antibiotic therapy for six weeks did not eradicate Brucella canis from affected bitches (as confirmed by blood culture and serology), it did prevent abortion. Five of the six bitches became bacteremic following treatment and serologic titers declined for a variable length of time. Abortion did not occur while these bitches were abacteremic - the number of live pups whelped and weaned by treated bitches was comparable with that in bitches before they became infected. However, the authors suggested that additional similar studies should be performed before a therapy regimen such as this could be recommended for general use. The authors noted that they had not investigated the risk of environmental contamination that would be present by maintaining infected (although treated) females in a kennel setting. Also, these investigators noted that the number of animals used in their study was small, and that their statistical analysis might therefore be misleading. Flores-Castro and Carmichael (reported by Pollock<sup>75</sup>) performed an extensive clinical trial using tetracyclines, trimethoprim and sulfadiazine (Tribrissen<sup>R</sup>: Burroughs Wellcome Co., Research Triangle Park, N.C., 27709), streptomycin, sulfadimethoxine, rifampin, and demeclocycline, alone and in various combinations at differing dosages for variable

periods of time. None of these regimens was consistently successful in eliminating the Brucella canis organism from infected dogs. However, another therapeutic regimen studied by Flores-Castro and Carmichael apparently eliminated the organism from 15 of 18 infected dogs. This therapy consisted of minocycline (12.5 mg/lb. b.i.d.) for two weeks, given together with streptomycin (10 mg/lb. b.i.d.) for the first week. However, as Pollock points out, the minocycline must be given at extremely high dosages, and the cost for the minocycline alone (for an 80 lb. dog) would be over \$500 (1979 prices). In addition, Pollock notes that the work by Flores-Castro and Carmichael involved experimentally infected animals, with treatment following experimental inoculation after a relatively short time period. Again, whether chronically infected animals in a natural setting would respond as did the experimentally infected animals is unknown.

It may be concluded that no program of antibiotic therapy has proven completely effective<sup>8,18</sup>, although success in individual cases has been achieved<sup>8</sup>. Claims for cures of canine brucellosis must be interpreted with caution<sup>75</sup>: negative blood cultures and a drop in antibody titer during or shortly following therapy do not prove that the organism has been eliminated from the animal. The Brucella canis organism, persisting in sequestered sites such as the prostate gland and lymph nodes, may proliferate after therapy ceases, and again become disseminated throughout the host's tissues and blood<sup>75</sup>. Brucella canis has been isolated from the lymph nodes of abacteremic, low titer dogs for as long as 13 weeks after intensive tetracycline treatment<sup>54</sup>. Other investigators feel that the ineffectiveness of

drugs on Brucella canis is due to the intracellular location of the organism in the host<sup>64</sup>.

Euthanasia is recommended for infected dogs that are to be used for commercial breeding purposes<sup>8,18,75</sup>. Euthanasia, although recommended for pet dogs<sup>8,18,75</sup>, is frequently met with opposition. The family which does not wish to euthanize the pet animal, has two options:

1. Isolation of the dog, awaiting spontaneous recovery<sup>8</sup>. This may take up to three years, and, as previously noted, the threat of transmission of the disease to other pets and humans is everpresent. Transmission of the organism probably can be interrupted by castration of males, or by spaying of females<sup>8</sup>.
2. Antibiotic therapy<sup>8</sup>. Whatever regimen is chosen, the treatment must be "heroic and sustained"<sup>28</sup>, and even then a cure is not guaranteed. Such therapy should be followed by periodic serologic and/or bacteriologic monitoring<sup>8</sup>. Castration of males and spaying of females is recommended<sup>8,18,75</sup>. It should also be noted that antibiotic therapy may mask the disease and aid in its spread by converting clinically affected, bacteremic, high titer dogs to undetectable chronic carriers<sup>54</sup>.

#### Prevention and Control

An effective, acceptable vaccine has not been developed for the prevention of canine brucellosis. Carmichael and Kenney<sup>12</sup> reported on attempts to immunize dogs with killed vaccines. Heat- and formalin-inactivated preparations of Brucella canis, suspended in saline

solution, or mixed with aluminum phosphate, aluminum hydroxide, or calcium alginate gels conferred little or no immunity on experimentally challenged animals. The only bacterins that stimulated resistance were those that consisted of Brucella canis organisms emulsified with Freund's type (water-in-mineral oil) adjuvant. Animals which received this type of bacterin attained at least temporary immunity: they failed to develop bacteremia following oral administration of  $10^9$  organisms one to three months after the final dose of bacterin had been given and they exhibited agglutination titers up to 1:250. However, this bacterin was unacceptable for general use, because vaccinated animals developed extensive swellings, sterile abscesses and, in most dogs there was sloughing of the skin over the inoculation site. Additionally, sloughed areas healed very slowly, taking up to one to two months for total recovery. Carmichael and Kenney<sup>12</sup> also found that good immunity (agglutination titers up to 1:1000) was provided by a commercial, killed Brucella abortus adjuvant vaccine (Duphovac: Phillips-Duphar, Amsterdam, Holland) prepared from a rough strain of Brucella abortus (45/20) that was found antigenically similar to Brucella canis. Like the Brucella canis-Freund's adjuvant bacterin, however, the Brucella abortus bacterin produced severe reactions at the site of inoculation, thus making it unacceptable also. Carmichael and Kenney<sup>12</sup> also performed immunization studies with a live variant of Brucella canis. The wild type of Brucella canis is mucoid (M), and produces viscous growth in broth. Carmichael and Kenney<sup>12</sup> selected a variant of this organism which produces uniform growth in broth (unless kept for two or three weeks), and designated it as smooth-mucoid (SM). The SM



variant, after 60 transfers in tryptose broth, exhibited reduced virulence for dogs. Dogs experimentally inoculated with a live vaccine made from the SM variant showed no signs of clinical disease, other than a minimal enlargement of lymph nodes. Pregnant females did not abort, and males failed to develop epididymitis or orchitis. There was no reversion of the SM variant to the M type in vivo, and SM organisms recovered from vaccinated dogs did not produce signs of illness when inoculated into other dogs. Although the SM variant proved to be low in antigenicity, and only low antibody titers were observed in vaccinated animals, these animals were immune to oral challenge with  $2 \times 10^9$  virulent (M) organisms for up to 14 months following vaccination with the SM variant. However, the live SM-variant vaccine was not acceptable for clinical use, because it produced a persistent bacteremia in males and females (with subsequent distribution of live organisms to many tissues of the body), and because it also caused extensive hyperplasia of lymphatic tissue.

"In the absence of an adequate vaccine, prevention of infection must rely on prevention of exposure to the organism"<sup>75</sup>. Thus, before a newly acquired dog is introduced into a commercial kennel or experimental animal colony, it should be subjected to a period of isolation and serologic/bacteriologic testing. The isolation facility must be physically separated from the area where other dogs are maintained and every precaution should be taken to assure that cross-contamination between the two facilities does not occur. It is generally recommended that the new animal be serologically and/or bacteriologically negative on two tests, 30 days apart, before removing the animal from

quarantine<sup>11,24,35,54,61,63,72,73</sup>. Paired samples (serum and/or whole blood) are obviously necessary, in order to prevent the incubatory animal from entering the colony<sup>11</sup>. Breeders are encouraged to mate their dogs only to animals which have been proven brucellosis-free by at least one slide agglutination test<sup>75</sup>. Semen in artificial insemination programs must come from proven brucellosis-free studs, since Brucella canis can be isolated from the semen of infected males<sup>11</sup>.

The principle of isolation coupled with repeated serologic-bacteriologic testing also applies to the newly acquired pet dog entering a household where other dogs already reside. Failure to do this could result in all dogs in the household contracting Brucella canis from the new arrival. The family would not suffer economically from such transmission as would a commercial breeder, unless they decided to treat all affected dogs. However, the family would undoubtedly suffer emotionally at the loss of their animals, if they opted for euthanasia rather than long term, expensive, possible fruitless therapy.

If preventive measures fail, and canine brucellosis becomes established in a kennel, it can only be controlled by the serologic/bacteriologic identification of and disposal of infected animals<sup>11,24,35,54,75,99</sup>. Hill et al.<sup>40</sup> reported on the control of canine brucellosis in a dog breeding colony housing 39 female beagles, greyhounds and pointers. Blood culture was determined to be the diagnostic method of choice. Serum agglutination tests were used also, because, even though agglutinating antibodies may not exist at diagnostic levels initially, they persist longer than bacteremia. Initial testing of the 39 females revealed 21 which

were positive for Brucella canis. Infected dogs were removed to a separate facility and retained for further study. Blood culture and agglutination tests were performed monthly, and an occasional positive animal was found during the next five months. The epizootic of canine brucellosis was thereby controlled, and no further Brucella canis infected animals were found in the colony. Moore et al.<sup>64</sup> reported on the eradication of Brucella canis from a colony of 265 male and female beagles. Infected dogs were identified by bacteriologic isolation of Brucella canis from blood and by presence of a serologic tube agglutinating titer. Based on the results of the first test, each dog was placed into one of three groups:

Infected - bacteriologically and serologically positive dogs which were immediately removed from the colony (initially 54 dogs in this group);

Suspect - bacteriologically negative, serologically positive dogs which were housed separately in disinfected quarters (initially 150 dogs in this group);

Negative - bacteriologically and serologically negative dogs which were housed separately in disinfected quarters, with procedures instituted to prevent contamination from other dogs (initially 61 dogs in this group).

Bacteriologic and serologic tests were repeated monthly for four consecutive months and again three months later. The last infected dog was removed on the fourth test. In all, six "Suspect" and three "Negative" dogs were added to the "Infected" group, bringing the total number of infected animals up to 63. Observation of the colony

plus bacteriologic culturing of selected bitches and offspring for an additional five months failed to result in an isolation of Brucella canis. Pickerill and Carmichael<sup>73</sup> reported on the eradication of canine brucellosis from two commercial breeding kennels. One kennel consisted of 600 brood bitches and 50 males housed individually in wire cages in four buildings. A serologic survey of these animals indicated that 20% of them had high levels of agglutinating antibody to Brucella canis. In addition, a number of abortions were reported to have occurred in this kennel, and reproductive efficiency had declined. By using a series of monthly tube agglutination tests, the authors identified animals positive for Brucella canis (titer of 1:100 or greater). Positive animals were promptly removed from the breeding colony to a separate facility for further study of the disease. In this manner, canine brucellosis was eradicated from the colony in four months (five agglutination tests, one month apart). Kennel 2 consisted of 120 brood bitches and 15 males housed in suspended cages in a single building. Initially, the owners of these dogs did not agree to test and eliminate animals from the breeding colony. Instead, they attempted to isolate infected dogs in the same building with noninfected dogs. They did this by placing aluminum panels between and behind all cages, extending six inches beyond the boundaries of each cage. Also, improved husbandry practices were implemented (e.g., disinfection of equipment, washing of hands between examination of bitches for estrus or pregnancy, etc.). Over a five month period utilizing this program, abortions continued to occur and additional dogs became infected. The owners then agreed to an eradication program suggested by Pickerill and

Carmichael<sup>73</sup>, in which each dog would be tested monthly by hemoculture and agglutination tests. Animals found positive by either test would be immediately removed from the colony. By using this program, brucellosis was eradicated from the kennel in only three months (four agglutination tests, one month apart). After eradication had been accomplished in both kennels, all additional dogs added into the breeding colonies were placed in isolation quarters until two negative agglutination tests were obtained at a 30-day interval. Dogs in both kennels remained seronegative and free from disease for more than two years (length of surveillance period) following completion of the program. The authors concluded that the use of serologic methods alone to identify *Brucella*-infected animals (Kennel 1), or the use of serologic and hemocultural methods (Kennel 2) are both effective in the elimination of canine brucellosis from kennels. However, even though laborious and initially more costly, the addition of hemocultural techniques to an eradication program makes the program more efficient (i.e., shorter time interval until *Brucella*-free status is attained).

Although bacteriologic and serologic testing of dogs, with segregation of infected from noninfected animals, is the backbone of an effective control program, strict hygienic procedures and careful husbandry practices cannot be ignored. Such practices include<sup>24,54</sup>:

1. Cleaning of the kennel daily, followed by disinfection with an iodine-base compound (Betadine<sup>R</sup>: Purdue Frederick Company, Norwalk, Connecticut, 06856) or a quaternary-ammonium compound (Roccal<sup>R</sup>: National Laboratories, Montvale, New Jersey, 07645).

2. Wearing of separate outer clothing, shoe covers and gloves in each section of the kennel, particularly between quarantined new additions and selected clean breeding stock.
3. Disinfecting of hands before each animal is examined for heat or pregnancy.
4. Preventing the reentry of a previously infected dog, a dog with a low titer, or a dog that has experienced a "spontaneous cure" into the kennel.

Because canine brucellosis has neither the public health significance of a disease such as bovine tuberculosis, nor the widespread economic impact of Brucella abortus, it is unlikely that a general eradication program will ever be instituted<sup>75</sup>. Although canine brucellosis may be eliminated in closed populations, such as confined pet dogs or a particular kennel, the practitioner should expect new cases of the disease to occur sporadically among his patients<sup>75</sup>.

#### Pathogenesis and Epidemiology

As Carmichael and Kenney<sup>12</sup> noted, thorough studies on the pathogenesis of canine brucellosis have not been reported. Therefore, the exact nature of the infectious processes initiated by Brucella canis in the dog can only be surmised on the basis of limited studies and by analogy to descriptions of brucellosis which have been well documented in other species. In their previous experimental studies, Carmichael and Kenney<sup>11</sup> observed that bacteremia commenced approximately one to three weeks after oral or intravaginal exposure and it commonly

persisted for many months (in one group of 12 dogs, six were still bacteremic one year after oral inoculation). Generally, the bacteremia did not appear to be intermittent; furthermore, females which became abacteremic did not become bacteremic again following parturition. Brucella canis organisms were located in the leukocyte portion of the blood of bacteremic dogs, and detectable levels of agglutinating antibody appeared several days following the onset of bacteremia. Although dogs examined two weeks after oral infection had organisms in various tissues throughout the body, growth was most abundant in tissues of the reticuloendothelial system (i.e., lymph nodes, spleen, liver) and, in sexually mature males, in the prostate gland and epididymides. Brucella canis was commonly isolated from the placenta in pregnant females; however, the uterus was not a site of abundant bacterial growth in the nongravid female. The investigators concluded that Brucella canis organisms probably invade the fetus via the placenta. In addition, because of the high concentrations of bacteria in amniotic fluids and the presence of leukocytes in the lumen of the stomachs and intestines of aborted pups, they postulated that infection of the fetus may also occur as a result of ingestion of amniotic fluid.

Carmichael and Kenney<sup>12</sup> hypothesized a scheme for the pathogenesis of canine brucellosis in the sexually mature dog. As with any infectious agent, Brucella canis requires a portal of entry. In this case, the portal of entry is mucous membranes of the oropharynx, genital tract, and perhaps the conjunctiva. Following entry into the body, the organism undergoes phagocytosis by leukocytes. The exact role and extent of activity of neutrophils and macrophages involved

in this stage are not clearly understood. Phagocytized Brucella canis organisms are able to survive and even multiply in the host's leukocytes. The bacterium then becomes localized in regional lymph nodes and continues to multiply (retropharyngeal lymph nodes with oral or conjunctival entry; inguinal and iliac lymph nodes with genital entry). After increasing in numbers, the organism enters the blood (leukocyte associated), and a bacteremic period averaging six to 18 months in duration ensues. Next occurs further multiplication of the organism in various tissues of the body, primarily the reticuloendothelial system (lymph nodes, spleen, liver) and the genital tract (placenta of pregnant females, epididymides and prostate of males). The next stage is the immunological response on the part of the host. Humoral immunity (immunoglobulin types, sequence of production) and cellular immunity are discussed in the following section. Subsequent to the immune response, Carmichael and Kenney<sup>12</sup> hypothesize a possible immunopathologic basis for some of the lesions observed on post mortem (arteritis, lymphadenitis, splenitis). At this point in the pathogenesis of the disease, the host begins to eliminate organisms in the urine, semen, uterine discharges, aborted fetal tissues, milk, etc. Finally, the sequence of events terminates in immunity for the host. The nature of the resistance as well as the duration of immunity require additional study and documentation.

Moore<sup>61</sup> reported experimental animals which exhibited bacteremia with durations ranging from 26 to 33 months, thus confirming the observation by Carmichael and Kenney<sup>12</sup> of extended periods of bacteremia. Carmichael<sup>8</sup> noted in a publication subsequent to his work with Kenney,



that, in some dogs, the extended period of bacteremia may actually be two or more intermittent episodes of bacteremia. Hall<sup>35</sup> observed that bacteremia can be demonstrated even though dogs are afebrile and do not seem to be ill, the infection proving lethal only to the aborted fetus.

Flores-Castro et al.<sup>26</sup> performed a study in which SPF beagles were inoculated by the oral-conjunctival route with various strains of Mexican isolates of Brucella canis. All of the experimental animals developed a bacteremia within three weeks that persisted for at least 14 weeks (duration of observation period). In addition, all animals developed Brucella canis agglutinating and precipitating antibodies within three weeks after the inoculations.

Serikawa et al.<sup>84</sup>, in a survey of 1,186 stray dogs from the Gifu and Shiga areas of Japan, made an observation which contrasted with some of the work of Carmichael and Kenney<sup>11</sup>. Serikawa et al.<sup>84</sup> noted that they were able to frequently isolate Brucella canis in high concentrations from the lumen of the uterus of nongravid females. The authors concluded that the nongravid uterus might play an important role as the site of infection eventually resulting in abortion.

Van Hoosier et al.<sup>94</sup> noted in a study of naturally infected dogs, that the co-existence of high antibody titers and bacteremia indicates that the agglutinins do not play a significant role in the elimination of the infection. The agglutinins may in fact (as suggested by Carmichael and Kenney<sup>12</sup>) contribute to the pathogenesis of the disease by immunological mechanisms. Vascular changes and glomerulosclerosis

may be suggestive of such a process. Furthermore, Van Hoosier et al.<sup>94</sup> observed that naturally infected male dogs in a breeding colony appeared to routinely develop no bacteremia, and yet did produce seroagglutinins. The authors believed that these results could indicate the occurrence of a brief, transient bacteremia rather than the persistent bacteremia reported by previous investigators.

### Immunity

The degree and duration of resistance, and the underlying mechanisms of immunity are not well understood for canine brucellosis. Generally, dogs which recover from a natural or experimental infection are resistant to subsequent attacks of illness by Brucella canis<sup>12,86</sup>. Carmichael and Kenney<sup>12</sup> reported that five dogs that had been inoculated orally with Brucella canis and maintained in isolation units for up to two years were immune upon reinoculation. Their immunity was challenged orally with  $10^8$  viable organisms at least three months after initial bacteremia had ceased and agglutinating antibody titers had decreased to 1:50 or less. None of the re-exposed animals developed bacteremia, nor did agglutinin titers increase significantly. The latter observation perhaps implies that, as with other Brucella, cell-mediated immunity is more important than humoral antibody<sup>63,66,75,94</sup>. Finally, in none of the five experimental animals reported by Carmichael and Kenney<sup>12</sup> could bacteria be isolated when tissues were examined bacteriologically at necropsy. Although females may abort sequentially up to three litters<sup>12,86</sup>. Carmichael and Kenney<sup>12</sup> also reported that tests

of sera from litters of pups from bitches that had apparently recovered showed that several of the pups had low levels of maternal antibody; however, none of the pups were bacteremic, nor in any other way appeared to be infected with Brucella canis.

Moore and Gupta<sup>63</sup> performed a study which also indicated that recovered animals have a degree of immunity against reinfection. These investigators used three groups of animals: recovered dogs - these animals had recovered spontaneously from infection, and had no significant titer (11 dogs); low titer dogs - these dogs had a persistent low titer since the time of original testing (12 dogs); and control dogs - these animals demonstrated no titer to Brucella canis (four dogs). All animals received an oral dose of from  $10^6$  to  $10^{10}$  Brucella canis organisms. Only one of the 12 dogs with low titers became infected. None of the other "low titer" dogs nor any of the "recovered" dogs ever became bacteremic. Three of the four "control" dogs developed bacteremia. Agglutinating titers for the animals remained stable, except for the one bacteremic "low titer" animal (titer increased), and the three bacteremic "control" animals (developed a titer). At the time of necropsy about 50 days post-inoculation, intensive bacteriologic culturing failed to result in the isolation of Brucella canis in any of the dogs, other than the one "low titer" animal and the three "control" animals.

Van Hoosier et al.<sup>94</sup> reported on the antibody response and mercaptoethanol sensitivity of agglutinins in naturally infected dogs over a 30 month period. Because the dogs were naturally instead of

experimentally infected, the time of infection was determined by either the date of abortion, date of initial positive blood culture, date of initial presence of seroagglutinins, or a combination of these factors. Therefore, the time of infection for dogs included in the study was an estimate, subject, the authors claim, to a two-week variation. The investigators observed that 80% (8/10) of the females were bacteremic at three months post-infection, 100% (10/10) at six months, 50% (5/10) at 18 months, and no bacteremic animals (0/10) were observed after 27 months. Blood cultures from males used for breeding purposes were routinely negative. At necropsy, extensive bacteriologic culturing was performed on tissues from five females in which the organism was no longer detectable in peripheral blood. All tissues were negative, except the spleen of one animal, indicating that host defensive mechanisms had generally cleared the body of Brucella canis organisms in previously infected animals. These findings also indicated that an occasional animal remains infected even though peripheral blood cultures have become negative. Seroagglutinins were detectable in 80-85% (9/11) of the dogs by three months post-infection, and in 100% (11/11) of the animals by six months following infection. The percent of animals with antibody remained high and was 77% (10/13) at 30 months. Geometric mean titers gradually decreased with time, from approximately 1:325 at six months post-infection to 1:70 at 30 months. Van Hoosier et al.<sup>94</sup> also performed studies on these dogs to determine whether the immunoglobulin class of antibodies could be correlated with the stage of infection of the animals. Van Hoosier et al.<sup>94</sup> attempted to confirm the work of other investigators<sup>2,77</sup>

who had previously reported that in humans and cattle infected with Brucella abortus, there is a correlation between the type of antibody and the stage of the disease. Generally, IgG (7S) or 2-mercaptoethanol resistant agglutinins are associated with the chronic forms of brucellosis in which the organism can be isolated from tissues, while IgM (19S) or 2-mercaptoethanol sensitive antibodies are associated with recovery from infection and the absence of demonstrable organisms. The 2-mercaptoethanol eliminates agglutination by IgM antibodies as a result of the splitting of disulfide bonds while the agglutinating activity of IgG antibodies is not seriously affected<sup>20</sup> (quoted by Van Hoosier et al.<sup>94</sup>). Van Hoosier et al.<sup>94</sup> utilized this difference in sensitivity to differentiate immunoglobulin classes in their experiment. Six out of ten dogs exhibited a response similar to that reported in humans and cattle infected with Brucella abortus, as noted above. The initial antibodies of the six dogs (three months post-infection) were predominantly IgM. At 18 months post-infection, the agglutinins were IgG, and at 24 months a transition back to IgM antibodies had occurred. Peripheral blood cultures were positive until about 19 months post-infection and subsequently negative. The other four animals did not fit this general pattern. They had IgG antibodies throughout the course of the disease, and there was no correlation with positive or negative blood cultures. One of these four dogs, for example, demonstrated predominantly IgG agglutinins at three months post-infection. At 11 months following infection, both IgG and IgM antibodies were present, even through peripheral

blood cultures were negative after nine months. However, Brucella canis was isolated from the spleen at necropsy 14 months post-infection. This observation suggested that the persistence of IgG antibodies after bacteremia may be correlated with chronic infection. Therefore, the presence of IgG or IgM antibodies in non-bacteremic dogs may be useful in differentiating completely recovered animals from chronically infected ones. Van Hoosier et al.<sup>94</sup> also notes that, should a live, avirulent vaccine be developed, the presence of IgM antibodies may be helpful in distinguishing between antibodies resulting from natural infection (IgG) and those resulting from vaccination (IgM), as has been demonstrated with Brucella abortus in cattle.

Morisset and Spink<sup>66</sup> reported the results from some of their investigations into the immune response in natural infections. Analysis of immunoglobulins in colostrum and serum revealed pure agglutinating 19S globulin and at least four separate 7S globulins. Sera from either uninfected or infected animals seemed to have little or no in vitro bactericidal action on Brucella canis. In vitro phagocytic tests with polymorphonuclear leukocytes in the presence of opsonins from uninfected and infected beagles revealed not only phagocytosis of the brucellae, but also a killing action.

Saegusa et al.<sup>80</sup> reported in a study of 945 stray and nonstray dogs, that dogs with high titers (1:640 or greater) had specific agglutinins which were usually resistant to 2-mercaptoethanol. Dogs with low titers (1:320 or less) probably had a predominance of non-specific agglutinins, which were 2-mercaptoethanol sensitive. The

authors concluded that the use of 2-mercaptoethanol during serological testing to reduce nonspecific activity might be reasonable, although some cases in the early phase of infection could be missed.

Ueda et al.<sup>92</sup> performed serologic studies of 15 beagles naturally infected in a breeding colony. These investigators confirmed what was noted by Van Hoosier et al.<sup>94</sup>; that is, a high titer of Brucella canis agglutinins persists for a long period in infected dogs. Even though the infections had occurred naturally, the investigators were able to ascertain by examining breeding records that all of the dogs had been infected for six to 12 months or more. The finding that the agglutinins of these dogs were 2-mercaptoethanol resistant is not incompatible with the observation made by Van Hoosier et al.<sup>94</sup>, namely, that 2-mercaptoethanol resistant antibodies (IgG) tend to predominate in chronic Brucella canis infections. In another part of their study, Ueda et al.<sup>92</sup> observed the effect of immunosuppressive agents upon provocation of chronic infection with Brucella canis. The main objective was to determine if small numbers of organisms, such as those which might survive treatment, would proliferate in an immunosuppressed dog. Twelve positive male dogs were used which were blood culture positive or had highly elevated levels of serum agglutinins. The animals had subsequently been treated with tetracycline or streptomycin. By the beginning of the provocation experiment, all dogs had a decreased serum titer and negative blood culture. The dogs were then divided into three groups of four dogs each: Group A received cortisone treatment, Group B received antilymphocyte serum (ALS) treatment,

and Group C was untreated controls. Blood culture and measurement of agglutinin titer were made at intervals following the treatments, and all dogs were sacrificed on day 47 of the experiment, and isolation of Brucella canis was attempted from various organs. None of the test animals developed bacteremia, nor did any of them demonstrate significant rise in antibody titer. Brucella canis was isolated from one dog in Group A (prostate, epididymis, lymph nodes), two dogs in Group B (prostate, lymph nodes), and one dog in Group C (prostate). The authors concluded that persisting Brucella canis infection seems to be refractory to this kind of stimulation.

Carmichael<sup>8</sup> noted that spermagglutinins were observed in both the serum and in the seminal fluid portion of ejaculates from infected males. Humoral spermagglutinins were found to be IgG immunoglobulins. They agglutinated normal sperm and immobilized living sperm in the presence of complement. These were detected approximately 13 weeks post-infection and the titer declined by week 50. The humoral response was interpreted as a consequence of the liberation of sperm antigens into the extratubular epididymis by migrating macrophages that contained phagocytosed sperm heads, rather than by simple leakage of sperm through tubules. Presumably, as part of the cell-mediated immune activity, the macrophages were activated and possessed enhanced nonspecific phagocytic activity, and were responding to the presence of abnormal sperm (bent tails, swollen midpieces, double tails, etc.) which resulted from the Brucella canis infection. Seminal fluid agglutinins were IgA immunoglobulins, and were interpreted as the result of a local antibody response. These IgA antibodies also agglutinated



normal sperm; however, they did not inactivate living sperm in the presence of complement. Highest seminal plasma IgA titers occurred during the stage of severe epididymitis. An additional seminal plasma immunoglobulin with cytophilic activity (IgG class) was found in the seminal plasma of infected, but not of noninfected dogs. Carmichael concluded in noting that the role of these immunological phenomena is unclear in the pathogenesis of male infertility. It is possible that infertility in the male, in part, is mediated by isoimmune reactions resulting from the heightened nonspecific phagocytic activity of inflammatory cells attracted to the sites of Brucella growth in the epididymis.

Finally, Pollock<sup>75</sup> made an interesting observation in dogs which were cleared of Brucella canis by antimicrobial therapy immediately returned to the susceptible state. While dogs which spontaneously become abacteremic are subsequently immune to reinfection, dogs cleared by antibiotic treatment were not solidly immune to rechallenge. Pollock notes that, if a practical treatment is developed, the owner will need to be advised that his animal can immediately recontract the disease.

#### Host Range Studies

The only animal known to be naturally infected with Brucella canis to any significant degree is the dog<sup>55,61</sup>. Although probably more cases of canine brucellosis have been observed in the beagle breed than in any other canine breed, the beagle is now considered to be no more susceptible than other dogs. As noted earlier, this

disease was first observed and studied in large breeding colonies of beagles, which accounts for the apparently high prevalence in this breed.

Carmichael and Bruner<sup>10</sup> studied the pathogenicity of Brucella canis in small laboratory animals, primarily guinea pigs, mice and rabbits. All three of these species were found to be susceptible to intra-peritoneal inoculation with Brucella canis, but there was no mortality. Limited studies indicated little difference in pathogenicity of the bacterium for these different species. Macroscopic lesions in most of the animal species included enlarged spleens, from which the organism could be isolated. Some of the male guinea pigs and rabbits showed discrete abscesses and adhesions of the epididymis and testes to the tunica vaginalis in affected organs. Pure cultures of Brucella canis were isolated from the epididymides of these animals. Lymph nodes were slightly enlarged. Microscopic lesions consisted essentially of diffuse granulomatous changes in organs rich in reticuloendothelial cells. These consisted of reticular-cell hyperplasia and accumulations of macrophages, epithelioid cells, and plasma cells, all present in variable numbers. The organism was isolated from the blood of mice and guinea pigs for four weeks following inoculation. All experimental animals developed agglutinins in their sera against the canine organism, ranging from a titer of 1:25 at one week post-inoculation to 1:800 at six weeks.

Pickerill<sup>72</sup> performed species susceptibility studies in a number of domestic animals. Nonpregnant swine, sheep, and cattle were found to be highly resistant to infection with Brucella canis by the

oral-conjunctival route. None of the animals became bacteremic, and the organism was not isolated from tissues taken at necropsy four to six weeks after inoculation. In addition, five pregnant sheep inoculated orally, subcutaneously, and by oral-conjunctival instillation failed to become actively infected. All ewes lambled normally and Brucella canis was not isolated from blood and tissues of ewes and lambs at the time of necropsy. Of 14 cats inoculated orally, three became bacteremic. While dogs normally develop bacteremia about three weeks following oral inoculation, the three cats which became bacteremic did not do so until at least nine weeks post-inoculation. Four of the cats were pregnant, and some of them developed bacteremia, but not until three to four weeks after the end of gestation. None of the pregnant females aborted, giving birth after a normal gestation period. Although most of the kittens died within 24 hours of birth, there was a similar mortality rate of kittens from noninfected, control cats. Tissues from two of the dead kittens from inoculated test cats were cultured, and both were positive for Brucella canis. A surprising observation was that even bacteremic cats did not develop significant agglutinating antibody titers. Titers of partial agglutination were 1:50 or less.

Hoff et al.<sup>41</sup> performed a serologic survey for Brucella canis in opossums and seven species of wild carnivores collected from five states. The 2-mercaptoethanol tube agglutination test was utilized, with titers of 1:200 or greater regarded as indicative of

of active infection. Results are as shown:

Sera Tested for Brucella canis Agglutinins,

By Species, from Five States

<u>Species</u>	<u># Positive/# Tested</u>	<u>% Positive</u>
Opossum	0/196	0
Raccoon	1/360	.3
Skunk	0/17	0
Bobcat	1/7	14.3
Fox, red	1/68	1.5
Fox, gray	0/15	0
Coyote	2/103	2.0
Wolf	<u>0/4</u>	<u>0</u>
Total	5/770	0.7

The authors quoted the results of an unpublished study, in which four of 222 (1.8%) raccoons from urban areas of two counties in Florida were seropositive for Brucella canis. This was contrasted with the results of the present study, in which one of 269 (0.4%) raccoons from rural areas of seven Florida counties was positive. Hoff et al.<sup>41</sup> noted that 75 of the coyote specimens were from one refuge in Texas, and that two (2.7%) of the specimens were positive for Brucella canis, while 16 (21.3%) were considered inconclusive (low titer). The authors quoted another study, in which two of 24 (8.3%) cottontail rabbits from the same refuge were reported to have titers against Brucella canis.

Hoff et al.<sup>41</sup> concluded that either Brucella canis or a closely related (cross-reacting) agent was active in wildlife populations in that area of Texas. Finally, the authors emphasized that bacterial isolation was not attempted in the five seropositive animals, and therefore caution must be exercised in the interpretation of these findings. The sensitivity of the agglutination test was originally adjusted for the testing of dog sera, and titers detected in other species may be related to causes other than actual exposure to Brucella canis antigen.

Randhawa et al.<sup>76</sup> conducted a serologic survey for Brucella canis in domestic cats. A total of 170 cats were tested: 114 strays from animal shelters in California and 56 nonstrays from an animal hospital in Texas. Results are as shown below:

Seropositive Reaction to Brucella canis in Domestic Cats

	Cats from animal shelters (% pos)	Cats from animal hospital (% pos)
Rapid Slide Agglutination Test	5.3	7.1
2-ME-TAT (titer 1:50 or more)	11.4	8.9
Brucellosis Card Test	0	0

One of the 114 (0.9%) cats from the animal shelters and five of the 56 (8.9%) hospitalized cats were seropositive by the 2-mercaptoethanol tube agglutination test at titers of 1:200 or greater. Out of the total of 170 cats, 18 (10.6%) were positive at a titer of 1:50 or greater by the 2-mercaptoethanol tube agglutination test. Of this 18, eight (44.4%) were positive at titers from 1:100 to 1:400,

while ten (55.6%) were positive at 1:50. This tendency toward higher titers is not consistent with the low experimental titers reported by Pickerill<sup>72</sup>. Randhawa et al.<sup>76</sup> observed that, because isolation of bacteria from seropositive animals was not attempted, the findings of this study should be interpreted cautiously. The authors made no attempt to compare the three test methods nor the results obtained by each test.

Percy et al.<sup>70</sup> reported on experimental Brucella canis infection in Macaque monkeys (Macaca arctoides). In this study, two monkeys (one male, one female) were each inoculated with  $10^{10}$  organisms via the oral and conjunctival route, two other monkeys (one male, one female) received the same dose intravenously, and a fifth animal (male) served as a noninoculated control. Blood samples were taken weekly for bacteriologic and serologic studies. Brucella canis was isolated from at least one weekly blood sample from each of the inoculated monkeys. The earliest positive blood cultures were obtained two weeks post-inoculation (one male, one female) and Brucella canis was isolated from the blood of one monkey (male) up to seven weeks post-inoculation. By the second week post-inoculation, all four inoculated monkeys had agglutinating titers (tube agglutination test) of at least 1:160. These titers increased to 1:1280 in the four monkeys by no later than the fifth week post-inoculation. The two females were necropsied at five weeks post-inoculation. Brucella canis was isolated from liver and kidney tissue of one female and from uterine tissue of the other. The two male experimental animals were necropsied at ten weeks post-inoculation - Brucella canis was not isolated from

any tissues. Gross post-mortem findings in three of the inoculated animals revealed nothing attributable to the inoculation. The fourth monkey, the female from which Brucella canis was isolated from the uterus, exhibited a moderately enlarged spleen, and the uterus contained five ml. of gray gelatinous material. Histopathologically, focal granulomatous lesions were sometimes observed in the liver, spleen and lymphoid tissue of inoculated monkeys. These lesions were similar to those seen in cases of human brucellosis due to other species of Brucella. The control animal had no significant serologic, bacteriologic or post-mortem findings. The authors concluded by noting that this study demonstrated the susceptibility of one species of subhuman primates to Brucella canis, and the possible danger entailed in human exposure to Brucella canis should be emphasized.

#### Public Health Significance

##### 1. Recognized cases

Since the first case of human infection due to Brucella canis was reported in 1967, there have been a total of 18 cases reported in the United States through 1978<sup>14,16</sup>. (No additional Brucellosis Surveillance Annual Summaries have been published by the Center for Disease Control since the 1978 Summary<sup>14</sup>). The 18 human cases are described in Table 2<sup>16</sup>. There have been no known deaths due to Brucella canis infection in humans<sup>75</sup>. Ten (55.6%) of the 18 cases occurred in persons with known animal exposure, six (33.3%) occurred in laboratory workers, and for two (11.1%) of the cases, data concerning the type

of exposure was not available. Analysis of Table 2 shows that, of the 16 cases where type of exposure was known, eight (50%) were associated with pet dogs, and eight were occupationally related (six laboratory workers, one veterinarian and one animal caretaker). Published case reports are available for six of the 18 cases (numbers 3,4,9-12 in Table 2). Data for the remaining 12 cases are summarized in Table 2.

Case #3<sup>15</sup> involved a 20-year-old, female laboratory technician who, on January 12, 1968, had accidental oral contact with Brucella canis while pipetting the organism. About three weeks later she developed a "grippe-like" illness, characterized by low grade fever, night sweats, malaise, and fatigue. Enlargement of the cervical lymph nodes occurred five weeks after her contact with the organisms. The lymph nodes became painful, and the patient had difficulty holding her head erect. A blood culture on March 1 was positive for Brucella canis. The specific agglutination titer was 1:100 by March 1, and 1:250 by April 10. Treatment with tetracycline, streptomycin, and sulfonamides was initiated on March 1. Symptoms improved after five days of treatment, fever was absent after ten days, and enlarged lymph nodes dissappeared after one month of therapy.

Case #4<sup>15</sup> involved a 23-year-old female worker in the same laboratory as Case #3, who experienced an identical episode of accidental contact with Brucella canis on June 3, 1968. The patient immediately began two weeks of oral tetracycline treatment. She remained asymptomatic, although her specific agglutination titer against Brucella canis reached 1:500 by late June.



Case #9<sup>88</sup> was probably the first well documented case of naturally acquired human infection with Brucella canis. The patient was a 23-year-old female with a history of rheumatic heart disease. She was hospitalized on March 4, 1970, complaining of fever (104° F), chills, headache, and sore throat. The patient was found to have enlarged cervical lymph nodes and pharyngitis; there was no hepatic or splenic enlargement. Blood cultures taken during the first week of hospitalization yielded Brucella canis. Serum obtained from the patient on March 5 and March 11 revealed specific agglutinating titers of 1:250. The patient was treated with ampicillin and streptomycin and responded well, becoming afebrile within eight days. Antibiotic therapy was continued for four weeks. One year after completion of therapy, the patient remained well. Blood cultures were negative, and the agglutination titer against Brucella canis was 1:25. The patient owned two dogs, a male German shepherd and a female mixed-breed dog. The German shepherd was positive for Brucella canis by blood culture on April 21, 1970, and serum at this time revealed a specific titer of 1:500. Presumably this animal was the source of infection for the patient. The female dog was negative bacterologically and serologically. Twelve family members had negative blood cultures and no serological evidence of infection with Brucella canis. The infected dog was treated for two weeks with tetracycline, streptomycin, and sulfisoxazole. However, after completion of therapy, blood cultures remained positive for Brucella canis, and the agglutinating titer was 1:200. The family refused further evaluation or therapy for the dog.

One year later they reported that the animal continued to appear well.

Case #10<sup>67</sup> was that of a 42-year-old male, hospitalized on July 2, 1972 with fever (101.2° F), shaking chills and headache. The symptoms had been occurring for one week. Physical findings other than inspiratory crepitation of the left lung were normal. Intravenous ampicillin was initiated on the first day of hospitalization, and the patient's temperature returned to normal within four days. Five blood samples obtained before intravenous therapy grew an organism that was identified in culture as Brucella canis. On July 10, the patient was discharged, having been placed on oral ampicillin therapy for ten days. On July 22, two days after ceasing oral treatment, the patient again felt ill and resumed taking ampicillin. He was readmitted with a temperature of 102° F and slight hepatic tenderness. The patient became afebrile throughout hospitalization, no antimicrobial therapy was given, and a single blood culture obtained during this admission was negative for Brucella canis. The patient was discharged on July 27. Agglutination tests for Brucella canis antibody were positive at a dilution of 1:200 on August 8 and 1:100 on August 29. The patient owned a 4 1/2-year-old female dog which had been spayed at age six months, and a male cat. Four members of the patient's family, four of the patient's co-workers, the patient's cat, and seven neighborhood dogs were all negative for Brucella canis serologically and bacteriologically. Brucella canis could not be isolated from the blood of the patient's dog. However, the dog had a specific agglutinating titer of 1:100 on August 8, and a negative titer on August 29. Because this pair of

serologic results was compatible with convalescence from infection, the dog was presumed to be the source of infection for the patient.

Case #11<sup>67</sup> was an 18-year-old male, admitted to a hospital on April 11, 1973. This individual had been experiencing a nonproductive cough, headache, weight loss, and low-back pain for two weeks, and the day before his admission he had fever and chills. Nothing outstanding was noted upon physical examination. On April 13, 14 and 15, the patient's temperature spiked to 102-104° F. He was given acetaminophen, but no antimicrobial therapy. The patient was discharged on April 17, continuing to take acetaminophen for a low-grade fever which persisted. When three blood cultures obtained on April 15 were reported to be positive for a Gram-negative rod (which later was identified as Brucella canis), he was treated at home with ampicillin. The patient experienced an apparent reaction to the ampicillin, characterized by a generalized rash. Therapy was stopped, and he was readmitted to the hospital on May 5. Physical examination revealed a generalized macular rash, conjunctival injection, pharyngeal erythema, and white ulcerated patches over the soft palate and pharynx. The liver and spleen were slightly enlarged. A blood culture on May 9 was positive for Brucella canis. Tetracycline was administered for one day, and then three doses of a preparation containing tetracycline, amphotericin B, and potassium metaphosphate (Mysteclin F<sup>R</sup>: E.R. Squibb and Sons, Princeton, N.J., 08540) were given on May 12 and 13. The patient's temperature returned to normal, and he was discharged on May 15. He took no antibiotics after discharge, and slowly recovered. Serum samples from two of the patient's friends and 31 neighbors were negative for Brucella canis

antibodies. Of 19 neighborhood dogs tested, two dogs (one of which was the patient's pet) had positive blood cultures for Brucella canis. These dogs and four others had Brucella canis agglutination titers of 1:200 or greater. Thus, approximately 30% (6/19) of the neighborhood dogs were positive for canine brucellosis. The patient's dog, presumably the source of his infection, was a female mongrel which was rarely confined. The dog had last delivered a litter in September 1972. In February 1973, the dog was in estrus and was seen being bred by a neighborhood dog. Subsequently, she appeared to have abdominal enlargement that went away; no litter was produced. No expelled tissues were noted by the patient. No Brucella canis isolates were recovered from cultures of bisected ticks which had been removed from the patient's dog and the other bacteremic animal.

Case #12<sup>4</sup> was a 48-year-old male who first sought medical attention in mid-December 1973 because he had been "feeling lousy" for about one week. He complained of chills and fever, decreased appetite and nausea. The patient had a medical history of hypertensive cardiovascular disease, residuals of an old cerebrovascular accident, and a positive tuberculin skin test. Physical examination revealed his known medical problems and a temperature of 100.2° F, but no other abnormalities were noted. For two weeks he was treated on an out-patient basis, during which time loss of appetite, nausea, weight loss and fever continued. He was examined again at the end of this two week period, and nothing abnormal was noted (specifically, there was no lymphadenopathy or hepatosplenomegaly). During a three day hospitalization period, the patient spontaneously became afebrile, felt better, and was discharged

without a specific diagnosis being made. A blood culture, obtained during the period of hospitalization, was positive for Brucella canis 12 days after discharge. No antimicrobial therapy was given. Three months later (March, 1974), the patient was again contacted. He had generally felt well during the interim and regained his appetite and weight, but had experienced relapses of fever and sweats about once a week. A blood culture on March 29 was negative, whereas another culture on April 2 was positive for Brucella canis. Empirical tetracycline therapy was initiated after the last positive culture. Laboratory data throughout the course of the illness, except for blood cultures, was unremarkable. During a subsequent epidemiologic investigation, blood samples for culture and serologic study were obtained from 13 household members and the family dog. Blood cultures from all 13 household members and the dog gave negative results. Serologic agglutination titers were 1:50 or less (non-diagnostic) for all family members. The patient at this time was found to have a specific agglutinating titer of 1:500, while the dog had a titer of 1:200. The dog, apparently the source of infection, was a 2-year-old mongrel which had borne two puppies about two months prior to the patient's onset of illness. One puppy was stillborn (the other died shortly after birth), and the patient had disposed of the remains. The dog was not overtly ill upon examination by a veterinarian during the epidemiologic investigation. The investigators inferred from this data a two month incubation period for this particular human case.

## 2. Clinical signs, physical findings, laboratory results

Generally, human infections with Brucella canis are very mild compared to cases of human brucellosis contracted from other domestic species<sup>3,75</sup>. A summarization of the clinical signs observed in human patients would include primarily mild fever, chills, malaise, headache, anorexia, weight loss, loose stools and sore throat<sup>4,24</sup>. One author describes the disease in humans as a nonspecific febrile illness resembling a viral upper respiratory infection. Physical findings have been minimal and include fever, adenopathy and occasionally splenomegaly<sup>4,24</sup>. In the nonlaboratory acquired infections there has been a minimum of adenopathy without splenomegaly<sup>4</sup>. Complications arising from other forms of brucellosis (e.g., meningitis, endocarditis, orchitis, suppurative splenitis, arthritis) have not been reported in Brucella canis infections<sup>4,8</sup>. All patients have shown a significant (1:100 or greater) tube agglutination titer, and Brucella canis was isolated by hemoculture from 13 of the 18 patients<sup>75</sup>.

## 3. Transmission

The route by which Brucella canis is transmitted from dogs to humans is not known. However, human infection is most likely accomplished by oral contact<sup>4</sup>. Infective aerosols, which play an important role in the transmission of other forms of brucellosis in abattoirs<sup>79</sup>, could also be important in the transmission of Brucella canis to humans (especially laboratory personnel)<sup>4</sup>.

## 4. Diagnosis

Due to the vague symptomatology in man and the inability of Brucella abortus antigen (which is used in routine brucellosis serologic tests<sup>4</sup>)

to react with sera containing Brucella canis antibodies, the diagnosis of the infection in man is difficult<sup>96</sup>. A definitive diagnosis depends upon identification of the organism by blood culture, a technique hampered by the fact that the pattern of bacteremia in humans is variable<sup>4</sup>. Serologic tests are also helpful in establishing diagnoses; in man, titers which are considered significant are 1:100 or greater<sup>4</sup>. The Center for Disease Control (Department of Health and Human Services) uses the following definitions of diagnosis<sup>14</sup>:

- a. Confirmed case - a clinical specimen culture-positive for Brucella (Brucella canis), or clinical symptoms compatible with brucellosis such as any combination of fever, sweats, chills, undue fatigue, anorexia, weight loss, ..., and a four-fold or greater change in Brucella (Brucella canis) agglutination titer between acute and convalescent serum specimens obtained two or more weeks apart and studied at the same laboratory.
- b. Presumptive case - clinical symptoms compatible with brucellosis with either a Brucella (Brucella canis) agglutination titer positive at a 1:160 or higher dilution on a single serum specimen obtained after the onset of symptoms or a stable Brucella (Brucella canis) agglutination titer positive at a 1:160 or higher dilution in serum specimens obtained after the onset of symptoms.

##### 5. Treatment

Human infections with Brucella canis have been successfully treated with ampicillin<sup>67</sup> or ampicillin plus streptomycin<sup>88</sup>. However,

tetracycline is the preferred drug for therapy of patients with either acute or chronic disease<sup>14,24,87</sup>.

6. Prevalence of *Brucella canis* in human populations

The results of seroprevalence surveys for *Brucella canis* in human populations are given in Table 3. The very high percentages of significant titers obtained by Monroe et al.<sup>60</sup> probably constitute a misrepresentation of actual conditions<sup>8</sup>. Monroe et al.<sup>60</sup> used a microtiter plate agglutination technique and accepted as significant the extremely low titer of 1:12. The results of Monroe et al.<sup>60</sup> are drastically different from those of any other investigators, implying that Monroe et al.<sup>60</sup>, though "well-intentioned", were "apparently inexperienced with a test procedure and its interpretation"<sup>8</sup>. Thus, in view of the generally low prevalence values obtained by other investigators, it seems that humans, like other non-canine species, are relatively resistant to the *Brucella canis* organism<sup>24</sup>. Obviously, however, the owners of infected dogs should be informed of the public health risk.



### III. Materials And Methods

#### Blood Specimen Collection

##### 1. Stray animals

Blood samples were collected from 200 stray dogs at the Franklin County Dog Pound from June through August, 1982. Following removal of the euthanized animals from carbon monoxide chambers, 10 ml. of blood were collected by cardiac puncture from each animal using a 12 cc. syringe and 1 1/2 inch-18 gauge needle. The blood was allowed to clot for three hours in sterile 10 ml. vacuum tubes. Samples were then centrifuged at 2800 r.p.m. for 15 minutes. The serum was removed and stored in sealed 12 X 75 mm. test tubes at -20° C. These 200 sera were later tested by the 2-mercaptoethanol tube agglutination test to determine the seroprevalence of Brucella canis-infected dogs in the stray population. Twenty of the blood samples from which serum had been removed were frozen at -20° C to produce hemolysis. The samples were thawed, recentrifuged at 2800 r.p.m. for 15 minutes, and an additional small amount (approximately 1 ml.) of serum was removed and stored. This hemolyzed serum was used to compare results of agglutination tests on matched pairs of hemolyzed/nonhemolyzed serum samples. In addition to collecting 10 ml. of whole blood for serological analysis from each animal, another 7 ml. of whole blood were collected (by the same procedure) from 20 of the pound animals and placed in sterile 7 ml. heparinized vacuum tubes. After centrifugation at 2800 r.p.m. for 15 minutes, the plasma was removed and stored in sealed 12 X 75 mm. test tubes at -20° C. This plasma was used to compare results of

agglutination tests on matched pairs of plasma/serum samples. For each animal from which a sample was obtained, the following information was recorded: breed, sex, approximate weight and age, and location of capture by animal control personnel.

2. Non-stray animals

Samples from all of these animals were tested by the 2-mercapto-ethanol tube agglutination test to determine the seroprevalence of Brucella canis-infected dogs in the respective populations.

a. College of Veterinary Medicine, Ohio State University

Randomly selected serum samples from 200 dogs treated at the small animal clinic during April through June, 1982, were obtained from the Department of Clinical Sciences. The samples were used by Ohio State University veterinary personnel for blood chemistry analysis. Unused portions were stored in 13 X 75 mm. plastic-capped test tubes at -20° C. Accompanying data collected for each case included clinic number, breed, sex, weight, age, residence, and admitting diagnosis.

b. Dublin Veterinary Clinic, Dublin, Ohio

Unused portions of plasma samples collected for routine heartworm testing during May and June, 1982, were obtained from 89 animals. The plasma was stored in sealed 10 X 75 mm. test tubes at -20° C. Data collected for each animal included breed, sex, weight, age, and residence.

c. Reynoldsburg Animal Hospital, Reynoldsburg, Ohio

Serum samples were collected from various dogs encountered in this practice from September, 1980, through March, 1982. The

samples were utilized in surveillance studies, such as for the prevalence of parvovirus in dogs. Ninety-nine of these samples were obtained from this source, and stored in 12 X 75 mm. and 10 X 82 mm. stoppered test tubes at  $-20^{\circ}$  C. Accompanying data included breed, sex, weight, age, and residence.

d. American Addition, Columbus, Ohio

A total of 62 serum samples, collected during June and July, 1981, in conjunction with a prevalence study for Rocky Mountain Spotted Fever, were used in the present survey. They were stored in 10 X 66 mm. stoppered test tubes and 15 X 60 mm. screw-capped vials at  $-20^{\circ}$  C. No demographic data was available for this population.

e. Vector-Borne Disease Unit, Ohio Department of Health

Twenty serum samples, submitted to the Ohio Department of Health for Rocky Mountain Spotted Fever testing during June and July, 1981, were utilized in this study. The samples represented dogs from various areas of the state of Ohio. The samples were stored in 12 X 75 mm. capped plastic test tubes at  $-20^{\circ}$  C. No demographic data was available for this population.

2-Mercaptoethanol Tube Agglutination Test (METAT)

1. Seroprevalence study for Brucella canis antibodies

Brucella canis concentrated antigen was obtained from the United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services Laboratory, Ames, Iowa (Serial Number 17701). Positive (1:3200) and negative reference sera were obtained

from, respectively, the Department of Clinical Sciences and Department of Veterinary Preventive Medicine, College of Veterinary Medicine, Ohio State University. All sera were screened at a dilution of 1:50. This was accomplished by placing .04 ml. of each serum sample into separate 13 X 100 mm. test tubes. To each tube was added 1 ml. of a formalized 3.5% saline solution containing 0.1 M mercaptoethanol, plus 1 ml. of a formalized saline solution of Brucella canis test-antigen. The tubes were then covered and incubated at 37° C for 48 ± 3 hours. Results were interpreted as complete, incomplete, or negative, based on the amount of clearing of the supernatant. Samples showing complete or incomplete agglutination at a dilution of 1:50 were retested at dilutions of 1:50, 1:100, 1:200 and 1:400. This was accomplished by adding, respectively, .04, .02, .01, and .005 ml. amounts of each serum sample to four separate test tubes. One ml. amounts of formalized 0.1 M mercaptoethanol and formalized test antigen were added to each tube, which were then incubated and interpreted as noted above. Animals which were positive (complete clearing) at a 1:400 dilution were retested, at dilutions of 1:800, 1:1600, 1:3200, and 1:6400. This was done by diluting each serum sample 1:16 with physiologic saline. Then, .04, .02, .01, and .005 ml. amounts of each diluted sample were placed in four separate test tubes, mixed with one ml. each of formalized 0.1 M mercaptoethanol and test antigen, and incubated/interpreted as previously noted. Positive and negative reference sera were used as controls. The test used in this survey was developed by the United States Department of Agriculture, Diagnostics Reagents Section, Veterinary Services Laboratory, Ames, Iowa. Serologic evidence of infection was considered to be indicated

by a titer of 1:100 or greater<sup>24</sup>, where titer means the highest dilution in which complete agglutination occurred.

2. Hemolyzed/nonhemolyzed serum comparison study

From each of 20 stray dogs, one hemolyzed and one nonhemolyzed serum sample was obtained as previously described. Each sample was tested at a dilution of 1:50 by the technique described for the screening of animals in the METAT seroprevalence study. Results of the pair of tests for each animal were tabulated so that a comparison of results (hemolyzed versus nonhemolyzed serum) could be made.

3. Plasma/serum comparison study

From each of 20 stray dogs, one serum sample and one plasma sample were obtained, as noted earlier. Each sample was tested at a dilution of 1:50 by the technique described for the screening of animals in the METAT seroprevalence study. Results of the pair of tests for each animal were tabulated for purposes of comparison (i.e., plasma versus serum results).

Agar Gel Immunodiffusion Test (AGID)

Ten serum samples were submitted to the Diagnostic Laboratory, New York State College of Veterinary Medicine, Ithaca, New York, for the purpose of AGID testing. Eight of the samples were selected from the various dog populations previously described, so as to include all sera that had shown complete agglutination by the METAT, plus other samples which had shown incomplete and negative METAT results. Two of the samples were the positive and negative reference sera used for the METAT. The AGID test was conducted blindly, so that a valid comparison of its results could be made with those of the RSAT and the METAT.

#### Rapid Slide Agglutination Test (RSAT)

A canine brucellosis diagnostic test kit was obtained from a commercial source (Pitman-Moore, Inc., Washington Crossing, N.J., 08560; Canine Brucellosis Reagent Serum, Canine Origin - serial number 219, expiration date September 7, 1985; Canine Brucellosis Agglutination Antigen - serial number 111, expiration date July 2, 1985; 2-Mercapto-ethanol 0.2 M Solution - serial number 306, expiration date August 13, 1984). The test was performed as recommended by the manufacturer using 50 serum samples. Ten samples were selectively chosen because they were previously tested by the METAT and AGID techniques. The remaining 40 samples were randomly chosen from the various dog populations previously described, and included sera that had shown incomplete and negative agglutination by the METAT. The RSAT was conducted as a blind study, so that a valid comparison of RSAT, AGID, and METAT results could subsequently be made.

#### Veterinary Diagnostic Services

Diagnostic services for two dogs identified as infected with Brucella canis were provided by the Departments of Pathobiology and Clinical Sciences, College of Veterinary Medicine, Ohio State University.

#### Determination Of Sample Size

The number of samples available from four of the canine populations was limited. Consequently, as many samples as were available were

procurred for the present study. The populations and respective sample sizes were as follows:

<u>Animal Population</u>	<u>Sample Size</u>
Dublin Veterinary Clinic, Dublin, Ohio	89
Reynoldsburg Animal Hospital, Reynoldsburg, Ohio	99
American Addition, Columbus, Ohio	62
Vector-Borne Disease Unit, Ohio Department of Health	20

The number of samples available from the other two populations (i.e., Franklin County Dog Pound; College of Veterinary Medicine, Ohio State University) were virtually unlimited, and therefore an appropriate determination of sample size was necessary. The following formula<sup>17</sup> was utilized in determining the sample sizes to be representative of these two populations:

$$n = \left[ \frac{Z_{\alpha} \sqrt{2\pi(1-\pi)} - Z_{\beta} \sqrt{\pi_t(1-\pi_t) + \pi_c(1-\pi_c)}}{\zeta} \right]^2$$

where:  $n$  = sample size of each population

$\pi_c$  = estimated prevalence in stray population

$\pi_t$  = estimated prevalence in cat population

$\zeta = \pi_t - \pi_c$

$Z_{\alpha}$  = upper  $\alpha$  percent of the normal distribution

$Z_{\beta}$  = lower  $\beta$  percent of the normal distribution

$\pi = (\pi_t + \pi_c)/2$

if:  $\pi_c = .08$ ;  $\pi_t = .01$  (based on previous serologic surveys of  
canine populations)

$$\alpha = .05$$

$$\beta = .10$$

then:  $\zeta = -.07$

$$\pi = .045$$

$$Z_\alpha = 1.96$$

$$Z_\beta = -1.28$$

By computation,  $n = 183$ . Thus, a sample of 183 animals should be procured from each population (Franklin County Dog Pound; College of Veterinary Medicine, Ohio State University). In actuality, 200 samples were collected from each of these two populations, to allow for error during collecting and testing of samples.



In summary, the number of animals sampled from each population is as indicated below:

<u>Animal Population</u>	<u>Sample Size</u>
Stray animals (Franklin County Dog Pound)	<u>200</u>
Non-stray animals	
College of Veterinary Medicine, Ohio State University	200
Dublin Veterinary Clinic, Dublin, Ohio	89
Reynoldsburg Animal Hospital, Reynoldsburg, Ohio	99
American Addition, Columbus, Ohio	62
Vector-Borne Disease Unit, Ohio Department of Health	<u>20</u>
	470
Total Animals Sampled	<u>670</u>

#### IV. Results

The results of the 2-mercaptoethanol tube agglutination test, used to estimate the prevalence of Brucella canis in various canine populations, are shown in Table 4. Estimated prevalence values for each population are as shown in Column e ("Percent Positive"). The overall (stray and non-stray) prevalence was 5/669, or 0.75%. The prevalence of Brucella canis antibodies in the stray population was estimated at 1.51%, while the prevalence in the combined non-stray population was 0.43%. Table 5 illustrates the results of the METAT by sex of the animals for each population. Columns e and f show the percent positive males and females for each population (when available). The overall (stray, College of Veterinary Medicine, Dublin Veterinary Clinic, Reynoldsburg Animal Hospital) percent positive males was 0.65% (2/307). The overall (stray, College of Veterinary Medicine, Dublin Veterinary Clinic, Reynoldsburg Animal Hospital) percent positive females was 0.77% (2/261). Table 6 shows the descriptive data (when available) for each of the dogs found positive by the METAT, along with corresponding METAT titers.

Results of the hemolyzed/nonhemolyzed serum comparison study are given in Table 7. As seen in this table, discrepancies between METAT results using hemolyzed serum and METAT results using nonhemolyzed serum occur for subject numbers 77, 96, 107, 119, and 132.

Results of the plasma/serum comparison study are shown in Table 8. Only one discrepancy (subject number 141) was noted between plasma METAT results and serum METAT results.

Table 9 illustrates the outcomes of the AGID tests which were conducted on nine of ten samples submitted. If complete agglutination at dilutions of 1:100 or greater is considered to be serologic evidence of Brucella canis infection (as stated in Section III - Materials And Methods), then there is complete agreement between the results of the METAT and those of the AGID test. One sample (number 7/Vector-Borne Disease Unit) could not be tested by AGID due to insufficient quantity, apparently resulting from leakage of the sample while enroute to Ithaca, New York.

Table 10 shows the results which were obtained using the RSAT (Pitman-Moore) to analyze 50 subjects for Brucella canis infection, along with the corresponding METAT results. Again, if evidence of infection is indicated by a METAT titer of 1:100 or more, and if the complete (without mercaptoethanol, with mercaptoethanol) RSAT is considered, then only two discrepancies are noted between METAT and RSAT results in Table 10. These discrepancies are one of the stray dogs (number 26) and one of the dogs (number 7) whose serum was obtained from the Vector-Borne Disease Unit. The negative RSAT results for these two animals may be considered to be "false negatives", with respect to the generally more accurate METAT results.

Table 11 summarizes the METAT, RSAT, and AGID test results for the ten subjects which were analyzed by all three methods. The negative RSAT result for stray dog number 26, already identified as a "false negative" result based on the corresponding METAT result, can be considered a "false negative" result also with respect to the corresponding AGID test result. The same cannot be said for dog number 7,

Vector-Borne Disease Unit, because the AGID test result for this animal was not available. All other subjects showed consistency among the three tests conducted on each.

One dog, whose serum was obtained from the College of Veterinary Medicine, was identified as having serologic evidence of Brucella canis infection (METAT titer 1:400). The owners of this animal were contacted, the situation explained to them, and euthanasia was recommended for the animal. The owners' predicament was complicated by the fact that they owned three additional dogs as well. The history, findings and disposition of each of these four dogs is as follows:

Clinic Case #186725: 7-year-old, 75 pound, male, German shepherd.

This was the animal identified during the canine brucellosis serosurvey. This animal, like the other three, was a farm-dog, allowed to roam freely. From September, 1977, through February, 1979, this dog was treated at the Ohio State University veterinary clinic for a number of disorders, including allergic dermatitis, otitis externa, nonspecific dermatitis, a mass on the dorsum of the back, lacerated forepaw, and the dog was hit by an automobile on two different occasions (the second incident resulted in prosthetic replacement of the left eye). In May, 1982, the animal was admitted to the Ohio State University veterinary hospital, during which time serum was drawn for routine analysis. A portion of this serum was used in the present study. Clinical signs upon admission included weight loss, decreased appetite, listlessness, and sores over both shoulders. Physical findings included fever (103° F), a heart murmur, palpable mandibular lymph nodes, and both

testes were descended, nonpainful, with the left somewhat larger than the right. Radiographs revealed an enlarged prostate, hip dysplasia, and spondylosis deformans of the lumbar spine. The animal was dismissed from the clinic before a definitive diagnosis could be made, with the owners declining further diagnostic efforts. The dog was not seen again until the owners returned the animal to the Ohio State University clinic for confirmatory serologic tests, after being notified as to the possible Brucella canis infection of their dog and advised of the public health risk. Serum obtained from the dog on November 16, 1982, and submitted to the Ohio State University Department of Clinical Sciences, was positive for Brucella canis agglutinins by the RSAT. The dog was allowed to return home, and the owners were advised to isolate it from other animals and humans. Meanwhile, further serologic testing was continued, and on November 18, the dog was found to be positive by the METAT, at a titer of from 1:400 to 1:800. At this point, even though a definitive diagnosis had not been made through isolation of the organism, the owners opted for euthanasia because of the numerous other medical problems this animal had demonstrated. The dog was admitted to the Ohio State University veterinary clinic and euthanized on November 24, 1982. Following euthanasia, blood was drawn by cardiac puncture for bacteriologic culture. Because a postmortem could not be performed for three days, tissues easily accessible (left and right epididymides, prostate) were also collected for bacteriologic culture. Hemolytic Staphylococcus was isolated from the blood - there was no growth of Brucella canis. Both epididymides were culturally positive for Brucella canis; the prostate was positive for hemolytic Staphylococcus and

Bacillus species, but not for Brucella canis. A postmortem was conducted on November 27, 1982, with the following results:

Gross lesions - The left epididymal head was prominent and had a granular appearance when incised. The prostate was symmetrically enlarged; variability in the texture of the organ was not evident on incision. There was irregularity of the ventral surfaces of the thoraco-lumbar vertebrae. L<sub>3-4</sub> and T<sub>13-L1</sub> were particularly involved. The cortex of the vertebrae could still be seen, with a proliferation of pale sclerotic, uneven bone ventrally. Irregular roughening of several of the thoracic vertebrae was also present. The inguinal and axillary lymph nodes were slightly enlarged. Visceral organs were autolyzed.

Pathologic anatomic diagnosis - Diagnoses included chronic lymphocytic epididymitis and orchitis, testicular atrophy, chronic pleocellular prostatitis, and coxofemoral dysplasia.

Etiologic diagnosis - Brucella canis epididymitis and orchitis.

Micropathology - The right and left testicles were altered by changes characterized by diffuse tubular atrophy, an absence of spermatogenesis, modest to large increases in intertubular collagenous connective tissue, upgrading of intratubular blood vessels, relative interstitial cell hypertrophy, and multifocal lymphocytic intertubular infiltrates, which were relatively rare. Surviving tubules were usually lined by Sertoli cells or were filled with amorphous hyaline debris. Focal interstitial

cell hyperplasia and a single intratubular Sertoli cell tumor were evident as well. The epididymal tubules were surrounded by dense fibrous connective tissue which was infiltrated by multiple foci of inflammation which consisted of lymphocytes, histiocytes, and plasma cells. The exudate was arranged in a peritubular pattern. Prostatic tissue was altered on a lobular basis. Changes were minimal in some lobules, while a dense pleocellular infiltrate distorted tubules in other lobules. Changes were focally quite severe, and consisted of modest to extensive increases in interstitial connective tissue, plasmacytic, lymphocytic, and occasionally neutrophilic interstitial cells, distortion and hyperplasia of prostatic tubule cells, and intraluminal neutrophilic infiltrates. Sections of liver, kidney, brain, skeletal muscle, heart and spinal cord were unremarkable. There was centrilobular acute pulmonary edema evident in the lung.

Clinic Case #222012: 13-year-old, 60 pound, female, German shepherd. This animal was first examined at the Ohio State University veterinary clinic on November 24, 1982. At this time a serologic test for Brucella canis was performed, because the other animal (#186725) described above from this household had shown serologic evidence of Brucella canis infection. A RSAT on this date was positive. Although the owners reported that this animal had shown a chronic vaginal discharge, no outstanding findings were noted upon physical examination. Because of the advanced age of the dog, and the high probability of Brucella canis infection in this animal, the owners decided to have it euthanized on this date. Blood, drawn by cardiac puncture following euthanasia, proved

to be positive for hemolytic Staphylococcus, but not for Brucella canis. Subsequent to the euthanasia of this animal, a METAT revealed a titer of 1:800 against Brucella canis. A postmortem was not conducted until November 27, 1982. Results were as follows:

Gross lesions - There was a one cm. smooth bordered polypoid mass suspended from the roof of the anterior vagina. The uterine mucosa was reddened and a small amount of mucoid material was present in the lumen. There were numerous 0.5 to 2.0 cm. cysts present on the left anterior uterine horn and salphinx; the cysts were filled with colorless or red, clear fluid. The ovaries contained follicles. There was moderated roughening of the ventral portions of some of the mid-thoracic vertebral bodies. The viscera were autolyzed. The inguinal lymph nodes were mildly enlarged.

Pathologic anatomic diagnosis - diagnoses included mild focal nonsuppurative endometritis, paraovarian cysts, and vaginal fibroma.

Etiologic diagnosis - The reproductive lesions were not gripping evidence of Brucella infection.

Micropathology - The vaginal mucosa covered a mass of loose fibrous connective tissue which did not involve deeper bundles of skeletal muscle significantly. Occasional clusters of small lymphocytes were present within the tissue. The endometrial stroma was edematous and considerable hemorrhage was present at some sites. The endometrial



glands were high columnar and showed a slight degree of coiling. A focal area of mononuclear inflammation was present and one of the larger venules was inflamed. Numerous follicles were present on the ovary. Numerous paraovarian cysts contained amphophilic material and were lined by a low cuboidal epithelium. Occasional clusters of xanthomatous macrophages were evident in the scant connective tissue wall of the cysts. Sections of kidney, liver, spleen, adrenal, heart, lung, and multiple sections of brain and spinal cord were unremarkable. Numerous plasma cells, many containing Russel bodies, were present in the medulla of the inguinal lymph node, together with active macrophages.

Tissue culture - A bacteriologic culture, prepared from an inguinal lymph node swab obtained during postmortem, was overgrown with swarming Proteus species and other bacteria.

Clinic Case #222016: age unknown, 21 pound, male, mixed-breed dog. This animal was a stray dog found by the current owners about one year before the present study. It was first seen at the Ohio State University veterinary clinic on November 24, 1982, when it was presented for Brucella canis serologic testing. Physical examination of the dog revealed hyphema of the right eye, a lesion consistent with previous reports<sup>81</sup> of Brucella canis infection in some dogs. A RSAT on this date was negative. The animal was sent home, and the owners were advised to have the dog reevaluated in six weeks. The dog was returned to the clinic on January 27, 1983. The left eye still appeared normal, while the cornea and lens of the right eye both exhibited cloudiness. An

extensive ophthalmologic examination was not performed. A RSAT on January 27 resulted in a weak positive reaction; it was recommended that the owners have the test repeated in one month. This situation was still pending at the time of this writing.

Clinic Case #218478: 7-month-old, 58 pound, male, German shepherd.

This animal was admitted to the Ohio State University veterinary clinic from June, 1982 through October, 1982 for routine vaccinations, external and internal parasite control, and for surgical correction of a tibial/fibular fracture resulting from being hit by an automobile. On November 16, 1982, this dog was returned to the clinic for serologic testing for Brucella canis. A RSAT on this date was negative. No additional information pertaining to this animal was available at the time of this writing.

## V. Discussion

The primary objective of the present study was to determine the prevalence of Brucella canis infection in various central Ohio canine populations by serologic technique (2-mercaptoethanol tube agglutination test). Our study indicated a pattern which had been reported in previous studies in the United States (Section II - Prevalence Studies In Various Geographic Regions); namely, that the prevalence of Brucella canis infection is higher in stray dogs than in non-stray dogs. The prevalence of Brucella canis infection in stray dogs was 1.51%, while the prevalence in non-stray dogs ranged from 0 to 0.5% in four of the five non-stray populations (Table 4). The fifth non-stray population, where serum was obtained from the Vector-Borne Disease Unit, Ohio Department of Health (V-BDU), appeared to be an exception, because a prevalence of 5.0% was determined for this group. However, this figure may be misleading for one or more of the following reasons:

1. The population at risk was very small (20 animals). Thus, even one positive animal could give a large prevalence value, which would not be statistically valid.
2. The one positive animal (number 7) detected in this population could have been a "false positive". It was designated as a "case" of canine brucellosis based solely on the results of the METAT. The titer of this animal by the METAT was 1:100, which was the minimum value in this study at which an animal was considered to have serologic evidence of Brucella canis infection. However, it is possible that this was a nonspecific

titer - neither blood nor other tissues were available for concomittant bacteriologic culture to confirm the presence of Brucella canis. As noted in Section IV - Results, an AGID test was not performed on serum from this animal (insufficient quantity of serum), and the RSAT gave a seemingly inconsistent, negative result.

3. This population of dogs may have been at a higher risk of contracting Brucella canis infection. All 20 of these dogs were animals which had been included in a survey for Rocky Mountain Spotted Fever, and a large percentage (71%) had been diagnosed serologically as having been exposed to the agent of Rocky Mountain Spotted Fever (Rickettsia rickettsii). As such, it was plausible that these animals were less controlled and confined than average pets, thus allowing them to wander into the environment of the rickettsial-carrying tick vector of Rocky Mountain Spotted Fever. Such wanderings could also allow these dogs to come into contact with other dogs, some of which were infected with Brucella canis.

If the animals from the V-BDU population are excluded, the prevalence of Brucella canis infection in non-strays was 0.22% (1/450). If the V-BDU population is included, the prevalence of infection in non-strays became 0.43% (2/470).

For purposes of further discussion, the V-BDU group data will be excluded from the general non-stray population data. This is justified by the fact that, as previously mentioned, this group of animals could have been at a higher risk of contracting Brucella canis infection than

the other non-strays, and, the single METAT-positive animal in this population was confirmed by neither RSAT nor AGID. Thus, the prevalence of Brucella canis infection in non-stray dogs in general in this study was considered to be 0.22%, while the prevalence in strays was 1.51%. As noted earlier, this high stray prevalence/low non-stray prevalence pattern was similar to that which had been reported by previous investigators in the United States. Although the magnitude of the prevalence values for strays and non-strays (1.51 and 0.22%, respectively) in this study was considerably lower than that reported by other investigators, the ratio of prevalence values for strays:non-strays was approximately 7:1 (1.51/0.22). This ratio compares favorably with that reported by other investigators. Brown et al.<sup>5</sup> reported a ratio of 9:1 for stray prevalence versus non-stray prevalence. Lovejoy et al.<sup>57</sup> reported stray/non-stray prevalences of 9.4 and 0%, while Galphin<sup>29</sup> reported respective prevalences of 7.6 and 0%. A direct comparison of the prevalence values derived by different investigators should be approached with caution, because, even if the investigators are using the same serologic test (e.g., tube agglutination), there may be variations due to lack of standardization of antigen, lack of concensus concerning significant titer, and other factors. Therefore, it may be more appropriate to compare the stray prevalence/non-stray prevalence ratio of one investigator with that of another, so that experimental variables would tend to "cancel" out.

Although the results of this study tend to indicate a high stray prevalence/low non-stray prevalence pattern in central Ohio, this cannot be "proven" statistically. Application of the Fisher's exact test

indicated that the two prevalence figures (1.51 and 0.22%) were not significantly different, based upon the number of animals sampled in the stray and non-stray populations. The Fisher's exact test calculations were as follows:

Null hypothesis: estimated prevalence in stray dogs is less than or equal to the estimated prevalence in non-stray dogs.

Alternate hypothesis: estimated prevalence in stray dogs is greater than the estimated prevalence in non-stray dogs.

		<u>METAT Result</u>		
		+	-	
<u>Population</u>	Stray	3	196	199
	Non-stray	1	449	450
		4	645	649

Using a one-sided hypothesis, the p-value for the given and all rarer configuration was:

$$p\text{-value} = \frac{199! \ 450! \ 645! \ 4!}{649! \ 3! \ 196! \ 449! \ 1!} + \frac{199! \ 450! \ 645! \ 4!}{649! \ 4! \ 0! \ 195! \ 450!}$$

$$p\text{-value} = 0.079488 + 0.008655$$

$$p\text{-value} = 0.088143$$

$$p\text{-value} \cong \underline{\underline{0.09}}$$

A p-value of 0.09 is not significant, and the null hypothesis was not rejected. Therefore, we could not statistically accept the alternate hypothesis that the stray prevalence value exceeds the non-stray prevalence value. However, we could intuitively accept the null hypothesis for several reasons:

1. Other previously cited studies in the United States indicated a high prevalence of Brucella canis infection in stray dogs relative to non-stray dogs. There are no known unique epidemiologic considerations with respect to environmental conditions, the Brucella canis organism, or canine populations in the state of Ohio which would preclude a similar prevalence pattern from occurring in central Ohio.
2. The calculated p-value of 0.09 is close to what would be considered a statistically significant value (i.e., "p" less than or equal to 0.05).
3. If larger sample sizes (stray and non-stray) had been used, a significant difference between prevalence values would probably have been obtained. The minimum sample sizes used in this study were calculated by the formula shown in Section III - Materials and Methods. This formula was based on the estimated difference in prevalence values between stray and non-stray populations. These estimates, based on previous serologic surveys of canine populations, were 0.08 and 0.01, respectively. The difference between these two figures, 0.07, is much larger than the difference between the stray and non-stray prevalence values estimated in this study for central Ohio. That is, the

stray prevalence (0.0151) minus the non-stray prevalence (0.0022) equals 0.0129. To detect such a small difference statistically would require the use of much larger sample sizes. Employing the same sample size formula as previously used, and based on a prevalence difference of 0.013, sample sizes for stray and non-stray populations would each consist of 1,045 animals. Problems and expenses encountered in a study of this magnitude would probably far outweigh the benefits derived from reducing the p-value from what was obtained in this study (0.09) to what is generally considered a statistically significant maximum value (0.05).

Previous studies<sup>47,53,84</sup> have indicated that there is no sex difference with respect to the prevalence of Brucella canis infection, and the present study supports this view (Table 5). Using the combined stray/non-stray data generated in this study, no significant difference was found between the prevalence of Brucella canis infection in males relative to females. The Fisher's exact test was employed in this analysis as follows:

Null hypothesis: estimated prevalence in males equals the estimated prevalence in females.

Alternate hypothesis: estimated prevalence in males does not equal the estimated prevalence in females.



		<u>METAT Result</u>		
		+	-	
<u>Population</u>	Males	2	305	307
	Females	2	259	261
		4	564	568

Using a two-sided hypothesis, the p-value for the given and all rarer configurations was:

$$p\text{-value} = \left[ \frac{307! \ 261! \ 4! \ 564!}{568! \ 2! \ 2! \ 305! \ 259!} + \frac{307! \ 261! \ 4! \ 564!}{568! \ 1! \ 3! \ 306! \ 258!} + \frac{307! \ 261! \ 4! \ 564!}{568! \ 0! \ 4! \ 307! \ 257!} \right] \times 2$$

$$p\text{-value} = [0.37137924 + 0.20956353 + 0.04402882] \times 2$$

$$p\text{-value} = [0.6249715] \times 2$$

$$p\text{-value} \approx \underline{\underline{1}}$$

This p-value was far from significant, and the null hypothesis was therefore not rejected. We concluded that there was evidence supporting the view that the prevalence of Brucella canis infection in males equals the prevalence in females.

Specific data concerning each of the dogs found positive for Brucella canis infection by the METAT is presented for informational purposes (Table 6). This descriptive data includes breed, age, and weight of each of the effected animals. It was not possible in the present study to analyze these variables in an effort to identify

potential predisposing factors of canine brucellosis. Such an analysis would require the use of a retrospective (case-control) study, in which each variable could be examined for its correlation to "cases" of canine brucellosis. Such studies (Section II - Predisposing Factors) have indicated that neither breed<sup>8,40,47</sup> nor age<sup>3,40</sup> influence the susceptibility of dogs to the Brucella canis organism. Similar studies concerning weight as a predisposing factor have not been noted in the literature. However, because weight is merely a function of other variables (including breed and age), it has not been viewed in itself as an important influencing factor in the epidemiology of canine brucellosis.

A number of investigators<sup>11,13,42,43,66</sup> have reported that only clear, nonhemolyzed serum should be used for Brucella canis serologic testing. Hemolyzed samples may cause autoagglutination<sup>75</sup>, thereby resulting in false positive reactions. A hemolyzed serum/nonhemolyzed serum comparison study was performed (Table 7) in order to observe what, if any, discordant results would occur if hemolyzed and nonhemolyzed serum samples from the same animal were subjected to the same serologic test (i.e., METAT). As seen in Table 7, there were five pairs of

discordant test results out of a total of 20 pairs of serum samples tested. The discordant pairs were:

<u>Subject</u>	<u>2-METAT Result (1:50 dilution)</u> <u>(hemolyzed/nonhemolyzed serum)</u>
77	$\pm/-$
96	$-/+$
107	$\pm/-$
119	$-/+$
132	$+/-$

The percentage of agreement between hemolyzed serum tests and nonhemolyzed serum tests was 75% (15 concordant pairs/20 total pairs). Autoagglutination apparently accounted for the three (77, 107, 132) discordant pairs of results in which suspicious ( $\pm$ ) 2-METAT results were obtained using hemolyzed serum, while negative results were obtained using nonhemolyzed serum. The other two (96, 119) discordant pairs of results consisted of negative 2-METAT results using hemolyzed serum, and positive results using nonhemolyzed serum. These apparently false negative reactions associated with the use of hemolyzed serum samples could have possibly resulted from the release of large amounts of intracellular materials during the process of hemolysis. The particulate matter could result in a flocculent-associated "cloudiness" in the test tubes, which would be interpreted as a lack of (negative) agglutination. It should be noted that, even though the degree of hemolysis in the hemolyzed serum samples was not quantified, the hemolysis present in these samples was visually much greater than any hemolysis which may

have been present in serum samples used in this study. Each of the populations surveyed had a number of serum samples with a slight degree of hemolysis present. As noted previously<sup>8</sup>, canine red blood cells are particularly fragile, and the collection of perfectly clear sera is very difficult to accomplish. Finally, if the percentage of agreement between extremely hemolyzed serum and nonhemolyzed serum samples approaches 75%, then it would seem likely that the use of slightly hemolyzed serum samples would result in relatively few false reactions.

The scientific literature has not indicated whether or not plasma samples are acceptable substitutes for serum samples in the various "serologic" techniques designed to detect Brucella canis-infected dogs. Because 89 of the 450 non-stray samples used in this study were plasma samples (Section III - Materials and Methods), a comparison study was conducted in order to ascertain whether plasma and serum samples from the same dog would produce consistent 2-METAT results (Table 8). As seen in this table, only one pair of plasma/serum samples out of a total of 20 produced a discordant outcome. Subject number 141 showed a negative 2-METAT result using a sample of plasma, and a suspicious result using serum. Thus, in this comparison study, the percentage of agreement was 95% (19 concordant pairs/20 total pairs). An inconsistency of 5% could easily be accounted for by "experimental error" (i.e., variation in the measurement of reagents, subjectivity of the visual determination of clearing of suspended particles in the tube agglutination test, et cetera). In view of the high percentage of agreement between

plasma and serum samples in this test group, it appeared appropriate to include the 89 non-stray plasma samples with the remaining non-stray serum samples used in this study.

Ten serum samples, previously tested for the presence of Brucella canis agglutinins by the METAT, were also tested by the AGID technique (Table 9). All METAT titers less than 1:100 were interpreted as negative serologic evidence of Brucella canis infection. AGID results of "positive" and "negative" were interpreted as meaning that Brucella canis antigen was or was not, respectively, detected in the serum sample; "partial identity" was interpreted as indicating either the early stages of a Brucella canis infection or the presence of cross-reacting heterologous antibodies<sup>71</sup>. The positive reference serum, in addition to having a clear precipitin line in the AGID test (positive reaction), also revealed a "spur", which indicated the presence of cross-reacting nonspecific antibodies. With respect to these interpretive guidelines, there was 100% agreement between the two serologic techniques (omitting sample number 7/V-BDU, which was not tested by AGID due to leakage during transit). In addition, these results were theoretically consistent with each other, in terms of the sensitivity and specificity of each test (i.e., the METAT is the more sensitive of the two techniques, while the AGID test is the more specific). Thus, sera presumably lacking Brucella canis agglutinins (negative reference serum, number 58/OSU, number 62/OSU) resulted in negative reactions by both the METAT and AGID. Sample number 5/OSU was suspicious by the METAT ( $\pm 1:50$ ), indicating either an early Brucella canis infection or the presence of nonspecific antibodies. The less sensitive AGID test,

however, failed to detect any antibodies in this sample, resulting in a negative reaction. Sample number 26/stray and number 159/stray, both positive at a titer of 1:200 by the METAT, showed only partial identity by the AGID technique. Sample number 183/OSU and number 79/stray both exhibited moderately high titers of 1:400 by the METAT. This was the lowest titer at which the AGID test gave an unequivocal positive result. The very high titer of the positive reference serum (1:3200) by the METAT also resulted in a clearly positive reaction by the AGID test. In addition, the more specific AGID technique detected cross-reacting heterologous antibodies in this serum sample.

Fifty serum samples, previously tested for serologic evidence of Brucella canis infection by the METAT, were also tested by the RSAT (Table 10). Shown in this table are six METAT-positive reactions (titer of 1:100 or greater), three METAT-suspicious reactions (titer of 1:50 or less), and 41 METAT-negative reactions (absence of agglutination at 1:50). One of the problems associated with the RSAT until only recently was the occurrence of a high percentage of false positive reactions, with Brown et al.<sup>6</sup> reporting a 58% false reactor rate. In the present study, the RSAT method without 2-mercaptoethanol reported seven sera as positive that were only suspicious or negative by the METAT. If the METAT is accepted as the standard of these two tests (METAT and RSAT)<sup>6</sup>, then the percent false positives by the RSAT in this study was 14% (7/50). However, the RSAT diagnostic kit (1982) includes 2-mercaptoethanol reagent for additional testing of samples in an effort to decrease the number of false positive reactions. The manufacturer recommends that all sera which are RSAT-positive be retested using the

2-mercaptoethanol. If both the RSAT and 2ME-RSAT tests are positive, the animal is presumptively diagnosed as being infected with Brucella canis, and a cultural examination of blood is recommended. If the animal is RSAT-positive, 2ME-RSAT negative, the animal is considered either in an early stage of Brucella canis infection, or the serum may contain nonspecific agglutinating antibodies to Brucella canis. The manufacturer suggests retesting the dog in 30 days by the 2ME-RSAT to distinguish between these two possibilities. Thus, in the present study, if complete RSAT results (without 2-mercaptoethanol, with 2-mercaptoethanol) are considered, then there were no RSAT-positive results that were reported as suspicious or negative by the METAT. That is, the percentage false positives became 0% (0/50). An unexpected finding in this study was the occurrence of two apparently false-negative results by the RSAT. Flores-Castro and Carmichael<sup>23</sup> and Brown et al.<sup>6</sup> both reported that the RSAT readily and accurately establishes the seronegative status of serum. In the present study, stray dog number 26 and V-BDU dog number 7 were both identified as positive by the METAT and negative by the RSAT. However, since the METAT results concerning V-BDU dog number 7 could not be confirmed by AGID, this animal was excluded from the following analysis, just as it and the other 19 V-BDU dogs were excluded from prevalence computations. Thus, if stray dog number 26 was considered to be the only animal that produced a false negative reaction by the RSAT, then a conservative estimate of the percentage of false negative reactions by the RSAT was 2.0% (1/49). If the tube agglutination test was considered the standard of these two serologic techniques, then Flores-Castro and Carmichael<sup>23</sup> reported a

false negative rate for the RSAT of only 0.2% (one RSAT false negative/411 total tests), while Brown et al.<sup>6</sup> reported a rate of 0.6% (15 RSAT false negatives/2367 total tests). The high percentage of RSAT false negative results in the present study could be explained by the small sample size (i.e., 49). A single false negative result, as was observed in this study, could result in an artificially high percentage of false negative reactions.

Ten subjects were each analyzed for the presence of serum agglutinins to the Brucella canis organism by the METAT, RSAT, and AGID serologic techniques (Table 11). As shown, agreement among the three techniques was complete, with the exception of sample number 26/stray (and also omitting sample number 7/V-BDU). The RSAT result for sample number 26/stray was previously identified as a false negative reaction, using the positive METAT result as a standard. This RSAT result was also a false negative when compared to the AGID result, as the latter technique indicated either an early Brucella canis infection or the presence of cross-reacting antibodies (i.e., partial identity). A chi-square analysis of the results obtained by these three serologic techniques was conducted as follows:

Null hypothesis: no difference among METAT, AGID, and RSAT results.

Alternate hypothesis: significant difference among METAT, AGID,  
and RSAT results.

#### Guidelines of interpretation

In a clinical situation, animals with suspect serologic results may be reevaluated after a specified time period, and changes



(increases/decreases) in titers noted. However, in an epidemiologic situation such as in the present seroprevalence survey, only one sample from each animal may be available, and each sample must therefore be judged as either positive or negative based solely on what is known about the serologic tests employed. Criteria for interpreting the METAT, AGID, and RSAT results, as they apply to the chi-square analysis, are shown below:

METAT

Positive result: titer of 1:100 or greater.

Negative result: titer less than 1:100.

AGID

Positive result: positive or partial identity reaction.

Negative result: negative reaction.

RSAT (without 2-mercaptoethanol/with 2-mercaptoethanol)

Positive result: +/+

Negative result: - or +/-

Based on these guidelines, the following chi-square table may be arranged from the data generated in this study:

		<u>Serologic Test</u>		
		METAT	AGID	RSAT
<u>Test Result</u>	+	5	5	4
	-	4	4	5

A chi-square calculation, employing Yate's correction, yielded a chi-square value of 0.053.

A significant chi-square value, at the 0.05 level with two degrees of freedom, is 5.991 or greater. The value derived in the present study (i.e., 0.053) was not significant, and the null hypothesis could not be rejected. Thus, we concluded in this study, that there was no significant difference among the three serologic techniques in the detection of Brucella canis agglutinins, when they were interpreted as noted above.

The clinical cases previously described represent four dogs with diverse characteristics, all owned by the same family, and which were ultimately presented to the Ohio State University veterinary clinic for diagnosis and/or treatment of various conditions. The history of these animals typifies the epidemiologic features of canine brucellosis introduced into a small population of dogs. The disease was insidious in nature, clinical signs were minimal and nonspecific, and the pattern of transmission of the disease was difficult to discern. The first of these four dogs identified as having serologic evidence of infection was Clinic Case #186725. This animal, a 7-year-old male German shepherd, had an extensive list of physical problems, as detailed in Section IV - Results. However, until identified as having an agglutination titer of 1:400 (by METAT) in the present study, the possibility of Brucella canis infection had not been included in the differential diagnosis of this animal. The manner in which this dog contracted the disease was unknown. All four dogs in this household were allowed to roam freely - if one of them introduced the disease to the others from an outside source or if

all four were exposed to a common infected animal was open to conjecture. The serum from Clinic Case #186725 which was used in the seroprevalence study was obtained from the animal on May 26, 1982, and had a METAT titer of 1:400. Another serum sample from this dog, obtained on November 16, 1982, was determined by the Ohio State University Department of Veterinary Clinical Sciences to have a METAT titer of from 1:400 to 1:800. In view of the stable agglutinating titer, and the fact that this dog was bacteriologically negative by blood culture and positive by tissue (epididymides) culture, it appeared likely that this dog was suffering from a long-term infection of Brucella canis (along with other unrelated and possibly related conditions). Abnormal findings (noted during clinical studies when the first serum sample was obtained) that might be attributed to Brucella canis infection included listlessness, decreased appetite and weight loss, palpable mandibular lymph nodes, testicles of different sizes, enlarged prostate, and vertebral lesions of the lumbar spine. While some of these symptoms were almost certainly due to infection by Brucella canis (e.g., enlarged prostate, testicular lesions), others could have resulted from infection by many other organisms (e.g., listlessness, decreased appetite, weight loss, enlarged lymph nodes). The diagnostic picture was further confused by the additional finding of sores over both shoulders, fever, heart murmur, and hip dysplasia. Pathologic findings following euthanasia of this animal were consistent with findings reported by other investigators. Gross lesions observed in this dog, including enlarged lymph nodes, prostate, and epididymis, have been reported by other investigators<sup>12,33,65,66</sup>. Histologic lesions seen in this dog and reported by

other investigators<sup>12,33,65,66</sup> included: testicular tubular atrophy with accompanying decreased spermatogenesis; destruction of prostatic glandular tissue with subsequent increase in connective tissue; cellular infiltration of epididymal, prostatic and testicular tissues by lymphocytes, neutrophils, and plasma cells. Micropathologic lesions of the liver, kidneys, and brain, although reported in other studies<sup>12,33,65</sup>, were not observed in this case.

After the first family pet was identified by serologic methods as a probable case of canine brucellosis, a second pet was brought to the Ohio State University veterinary clinic for serologic testing. This second pet, Clinic Case #222012, was a 13-year-old female German shepherd. As noted in Section IV - Results, this female was RSAT-positive on November 24, 1982, and serum obtained on that date was determined three days later to have a titer of 1:800 (by METAT). Paired serum samples could not be obtained, and the stage of infection was therefore difficult to estimate. However, a lack of bacteremia (a single blood culture was negative) in the presence of a moderately high titer indicated that this was either a chronically infected or convalescent animal. Tissues were not available for bacteriologic culture, and it could therefore not be ascertained if this dog actually harboured the Brucella canis organism. Physical examination of this animal on November 24 resulted in no outstanding findings, which is to be expected in the nongravid female<sup>12</sup>. Because of advanced age, the positive RSAT, and the presence of a probable Brucella canis-infected dog in the household, the owners elected to have Clinic Case #222012 euthanized on November 24. Pathologic findings following euthanasia did not provide

overwhelming evidence of Brucella canis infection. A vaginal fibroma and paraovarian cysts, although present, were not considered related to canine brucellosis. Moderate roughening of the ventral portions of some thoracic vertebral bodies bore little resemblance to reports<sup>39,48</sup> of discospondylitis in Brucella canis-infected dogs. The presence of mucoïd material in the uterine lumen and the presence of enlarged inguinal lymph nodes, although consistent with reports from some canine brucellosis investigators<sup>11,33</sup>, were by no means pathognomonic for this disease. Histologically, few genital lesions directly attributable to Brucella canis infection were noted. This observation has been previously reported<sup>33</sup> in nongravid dogs, and was considered to be a reflection of the nongravid state. The only micropathologic lesion of the genital system noted in this animal which could have been caused by a chronic Brucella canis infection was an edematous, hemorrhagic endometrial stroma, with endometrial glands exhibiting a slight degree of coiling. This condition was similar to that described by Carmichael and Kenney<sup>12</sup>. Histologic examination of an inguinal lymph node revealed an infiltrate of plasma cells and macrophages, particularly in the medullary region. This observation has been noted in previous studies<sup>12,33,66</sup>. Many of the plasma cells contained Russel bodies, which are distinct hyaline spheres located in the cytoplasm. These bodies are known to be secretory or degenerative products of the cell which occur in cases of chronic inflammation, and are believed to consist of gamma-globulin. Histologic lesions involving the kidney, liver, spleen, lung, and brain, although reported by other investigators<sup>12,33,65</sup>, were not observed in this animal.

The third dog owned by this family, Clinic Case #222016, was a male, mixed-breed dog, of undetermined age. This animal was a former stray, having been owned by this family for about one year before it was first presented to the Ohio State University veterinary clinic on November 24, 1982. On this date, in conjunction with the first two clinic cases, this dog was tested for Brucella canis exposure by the RSAT, and found to be negative. At the same time, the dog was found to be clinically normal, except for hyphema of the right eye. Two months later the dog was returned to the clinic for reevaluation. The cornea and lens of the right eye were both somewhat opaque, and a second RSAT produced a suspicious result. Saegusa et al.<sup>81</sup> reported ocular lesions of this sort in two of three experimentally infected beagles. One of the beagles experienced four recurrent episodes of bilateral corneal opacification over a 382 day period. The other beagle experienced two episodes of corneal opacification in only the left eye, followed by hyphema of the same eye, all over a period of 385 days. It seemed highly probable that the ocular lesion observed in Clinic Case #222016 was the result of Brucella canis infection. However, a point of inconsistency existed. The Brucella canis-infected dogs reported by Saegusa et al.<sup>81</sup> both had substantial serum agglutinin titers (up to 1:1280) at the time that they were exhibiting ocular lesions. Clinic Case #222016 demonstrated negative or only weakly positive serologic results during the time that ocular lesions were present. Perhaps the hyphema present in Clinic Case #222016 was caused by another unsuspected agent (e.g., trauma). False negative reactions are known to occur with the RSAT, and this could explain the first negative RSAT for this animal.

However, if Clinic Case #222016 had a substantial serum titer (as did the animals in the study by Saegusa et al.<sup>81</sup>), it would have been highly improbable to observe a negative RSAT followed by a weakly positive RSAT reaction. Another possible explanation would be that this animal was to some degree immunologically incompetent, and the humoral antibody response somewhat delayed. This would explain how Brucella canis-induced hyphema could be observed in the presence of a negative RSAT test. In addition, the second, weakly positive RSAT test would be indicative of a slowly responding immunologic system. This case cannot be fully explained without the performance of additional tests. For example, determination of further serum agglutinin titers and aqueous fluid agglutinin titers would be helpful, as would the culture of blood, aqueous fluid, and perhaps lymph node aspirates. Following the second, inconclusive RSAT result, the clinician recommended the owners have this dog reevaluated in one month - this case was still pending at the time of this report.

The fourth pet dog, Clinic Case #218478, was a 7-month-old, male German shepherd, which was negative for Brucella canis agglutinins by RSAT on November 16, 1982. This dog, being a young, prepubertal animal, had experienced fewer opportunities for encountering the agent of canine brucellosis. Thus, the negative RSAT could be an accurate reflection of the health status of this animal. The owners were particularly concerned about the health of this dog, because they wished to use this animal for breeding purposes. Consequently, when informed of the infectious nature of this disease, they immediately instituted preventive measures to

ensure that this dog would remain Brucella-free. No further information was available at the time of this report.



## VI. Summary

The prevalence of Brucella canis-infected dogs was estimated by the 2-mercaptoethanol tube agglutination test (METAT) to be 1.51% in stray animals, and 0.22% in non-stray animals. The prevalence ratio of 7:1 (1.51/0.22) was comparable to that reported by other investigators. Although the difference between the two prevalence values of 1.51 and 0.22% was not significant by the Fisher's exact test, the data indicates that the stray prevalence would be greater than the non-stray prevalence. The prevalence of Brucella canis infection in males (0.65%) was determined by the Fisher's exact test to be not significantly different from the prevalence in females (0.77%).

The percentage of agreement between the METAT using hemolyzed serum and the METAT using nonhemolyzed serum was 75%. A 95% agreement was observed between plasma METAT results and serum METAT results.

A chi-square analysis revealed that, in this study, there was no significant difference among three serologic techniques (2-mercaptoethanol tube agglutination test, rapid slide agglutination test, agar gel immunodiffusion test) in determining serologic evidence of infection with the Brucella canis organism.

Finally, four dogs in a single household were identified as Brucella canis-infected or potentially infected animals. The organism was isolated from tissues of one of the four dogs, and a pathologic examination was performed on this animal plus one of the remaining three dogs. The history of these animals represented a typical pattern of transmission of this disease in a small canine population.

Table 1: Seroprevalence Of Canine Brucellosis In Dogs

Area	Year	Significant Titer*	Group sampled/ % Significant titers
Alabama <sup>53</sup>	1972	1:100	Stray+non-stray 18.7
		1:200	Stray+non-stray 4.4
Florida <sup>42</sup>	1974	1:200	Stray 3.65
Tennessee <sup>27</sup>	1974	1:200	Stray 6.6
			Non-stray 1.9
Tennessee <sup>57</sup>	1976	1:100	Stray 9.4
			Non-stray 0
Georgia <sup>5</sup>	1976	1:100	Stray 9.0
			Non-stray 1.0
Georgia <sup>97</sup>	1977	1:100	Urban stray 9.0
			Rural stray 3.0
Mississippi <sup>29</sup>	1977	1:200	Stray 7.6
			Non-stray 0
Louisiana <sup>47</sup>	1980	1:200	Stray 12.3
Michigan <sup>91</sup>	1980	1:200	Urban stray 8.6
			Suburban stray 5.7
Japan <sup>93</sup>	1974	1:100	Stray 2.9
			Non-stray 4.7
Japan <sup>38</sup>	1977	1:160	Stray 3.0

Table 1: (continued)

Area	Year	Significant Titer*	Group Sampled/ % Significant Titers
Japan <sup>84</sup>	1977	1:320	Stray 2.8
Japan <sup>80</sup>	1978	1:640	Stray 1.9**
			Non-stray 2.6**
Mexico <sup>25</sup>	1976	1:100	Stray 28.0
Mexico <sup>26</sup>	1977	1:200	Stray 11.8**

\* tube agglutination test or 2-mercaptoethanol tube agglutination test

\*\* percent positive at indicated titer (or higher) or detection of Brucella canis organism in tissues

Table 2: 18 Cases Of Human Brucella canis Infection In The United States, 1967-1978

#	State/Year	Age/Sex	Type of Exposure	Signs and Symptoms	Method of Diagnosis
1	AR/67	60/M	3 or 4 hunting dogs-not examined.	NA*	<u>B. canis</u> recovered from either a retroperitoneal abscess or clotted aortic bifurcation surgical graft during repeat surgery.
2	TX/68	NA/M	Animal caretaker in kennel with infected dogs.	Asymptomatic	Seroconversion: less than 1:50 to 1:100.
3	NY/68	20/F	Laboratory	Fever, fatigue, night sweats, headache, depression, and painful cervical lymphadenopathy.	Blood culture positive. Seroconversion from 1:25 2 months before exposure to 1:250 12 weeks after.
4	NY/68	23/F	Laboratory	Asymptomatic	Seroconversion: less than 1:25 to 1:500. Culture negative.

Table 2: (continued)

#	State/Year	Age/Sex	Type of Exposure	Signs and Symptoms	Method of Diagnosis
5	MN/69	32/M	Laboratory	Headache, weakness, fatigue, sweating, chills, painful extremities, epistaxis, lymphadenopathy, and splenomegaly.	Blood culture positive. Single titer 1:320.
6	MN/69	52/F	Laboratory	Headache and malaise.	Titers 1:320 and 1:160.
7	MN/70	55/M	Laboratory	Fever, headache, and weakness.	Titers 1:320 and 1:640. Blood culture negative.
8	MN/70	24/F	Laboratory	Fever, weakness and malaise.	Blood culture positive. Single titer 1:640.
9	PA/70	23/F	Pet male dog culturally and serologically positive (1:500).	Headache, fever, chills, sore throat, and lymphadenopathy.	Blood culture positive. Single titer 1:250.

Table 2: (continued)

#	State/Year	Age/Sex	Type of Exposure	Signs and Symptoms	Method of Diagnosis
10	FL/72	42/M	Titer of pet spayed bitch dropped from 1: 100 to less than 1:50 when exam- ined after pa- tient's onset.	Fever, chills, and headache.	Blood culture positive. Titers 1:250 and 1:100.
11	TN/73	18/M	Pet adult bitch and neighbor's dog culture pos- itive.	Non-productive cough, headache, low-back pain, chills, fever, and weight loss.	Blood culture positive. Titers 1:1600 to less than 1:50.
12	TX/73	48/M	Pet bitch had de- livered stillborn litter 2 months prior to patient's onset. Seven months later, ser- opositive but cul- ture negative.	Chills, persistent fever, sweating and weight loss.	Blood culture positive. Single titer 1:500.

Table 2: (continued)

#	State/Year	Age/Sex	Type of Exposure	Signs and Symptoms	Method of Diagnosis
13	DC/74	2/M	NA	Hospitalized in an unresponsive condition with weakness, fever to 105F, constipation, and a history of epistaxis, vomiting and convulsions.	Blood culture positive.
14	LA/74	24/M	NA	Acute onset of constant fever, intermittent chills, headache, anorexia, cough, earache, and transient hemoptysis and neck stiffness.	Blood culture positive.
15	TN/74	34/M	Small animal veterinarian.	NA	Blood culture positive.
16	OK/74	45/M	Two of 3 pet dogs seropositive; third 1:80.	Insidious onset of intermittent fever, night sweats, body ache, weakness and weight loss.	Titers 1:80 and 1:160.

Table 2: (continued)

#	State/Year	Age/Sex	Type of Exposure	Signs and Symptoms	Method of Diagnosis
17	MI/76	55/F	Pet bitch delivered stillborn litter prior to patient's onset. Later shown to be seropositive.	Fever, weakness, anorexia, and hepatosplenomegaly.	Blood culture positive.
18	GA/76	22/M	Pet bitch aborted 2 weeks prior to patient's onset. Later was seronegative.	Fever, chills, sweating, and headache.	Blood culture positive.

\* not available



Table 3: Seroprevalence Of Brucella canis In Humans

Area	Year	Diagnostic Test/ Significant Titer	Group Sampled/ % Significant Titers	
Texas <sup>58</sup>	1970	TAT <sup>*</sup> /1:50	Canine colony personnel (1/27)	3.7
** 55 NA (1208 mili- tary recruits presumably from various geographic regions)	1973	TAT/1:100	Male military recruits (5/1209)	0.4
Florida <sup>42</sup>	1974	TAT/1:200	Animal shelter workers (1/167)	0.59
			Veterinarians (0/43)	0
Florida <sup>43</sup>	1975	TAT/1:200	Residents (1/303)	0.3
Oklahoma <sup>60</sup>	1975	MPAT <sup>*</sup> /1:12	Newborn infants (11/193)	5.7
			Persons with average exposure to dogs (1374/2026)	57.8
			Males (567/913)	62.1
			Females (806/1113)	52.4
			Veterinarians (53/73)	72.6
			Male blood donors (210/369)	66.9

Table 3: (continued)

Area	Year	Diagnostic Test/ Significant Titer	Group Sampled/ % Significant Titers	
Argentina <sup>95</sup>	1979	GD* /not applicable	Corrientes Province (21/1065)	2.0
			Neuquen Province (2/387)	0.2
Mexico <sup>25</sup>	1976	PAT*/1:100	Residents (27/203)	13.3
Japan <sup>99</sup>	1974	NA	Colony and laboratory personnel (0/11)	0

\* TAT=tube agglutination test

MPAT=microtiter plate agglutination technique

GD=gel-diffusion

PAT=plate agglutination test

\*\* not available

Table 4: Seroprevalence Survey For Brucella canis By Canine Population As Determined By METAT \*\*\*

Source	a. Total Animals	b. Number Negative *	c. Number Suspicious *	d. Number Positive *	e. Percent Positive (d/a)
Franklin County Pound	199**	162	34	3	1.51
Non-stray Animals					
College Vet Med	200	192	7	1	0.50
Dublin Vet Clin	89	87	2	0	0
Reynold An Hosp	99	95	4	0	0
American Addition	62	61	1	0	0
Vet-Borne Dis Unit	20	18	1	1	5.00
Total	669	615	49	5	

\* negative=absence of agglutination at 1:50

suspicious=incomplete agglutination at 1:100 or lower

positive=complete agglutination at 1:100 or higher

\*\* one sample from this population could not be used because no serum was present after centrifugation

\*\*\* 2-mercaptoethanol tube agglutination test

Table 5: Seroprevalence Survey For Brucella canis By Population And Sex As Determined By METAT\*\*\*

Source	a. Number Males	b. Number Females	c. # Positive Males	d. # Positive Females	e. % Positive Males (c/a)	f. % Positive Females (d/b)
Franklin County Pound	117	82	1	2	0.85	2.44
Non-stray Animals						
College Vet Med	107	93	1	0	0.93	0
Dublin Vet Clin	48	41	0	0	0	0
Reynold An Hosp	35*	45	0	0	0	0
American Addition	NA**	NA	0	0	0	0
Vec-Borne Dis Unit	NA	NA	<u>1</u>	<u>0</u>	NA	NA
Total	3	3		2		

\* sex of animal not available for 19 subjects

\*\* not available

\*\*\*2-mercaptoethanol tube agglutination test

Table 6: Dogs Seropositive For Brucella canis As Determined By METAT\*\*\*

Source	Breed	Sex	Age(yrs)*	Weight(lbs)	METAT Titer
Franklin County Pound					
#26	German Shepherd	M	3	40	1:200
#79	Terrier Mix	F	1	15	1:400
#159	Chihuahua Mix	F	2	10	1:200
College Vet Med					
#183	German Shepherd	M	7	75	1:400
Vec-Borne Dis Unit					
#7	NA**	M	NA	NA	1:100

\* estimated by dentition for strays

\*\* not available

\*\*\*2-mercaptoethanol tube agglutination test

Table 7: Hemolyzed/Nonhemolyzed Serum Comparison Study Using The METAT\*\*

Subject	METAT Results* (Hemolyzed/Nonhemolyzed Serum)
62	-/-
64	-/-
65	-/-
75	-/-
77	±/-
81	-/-
86	-/-
88	-/-
93	-/-
96	-/+
102	-/-
107	±/-
112	-/-
113	-/-
119	-/+
128	-/-
130	-/-
132	±/-
142	-/-
144	-/-

\* 1:50 dilution

\*\*2-mercaptoethanol tube agglutination test

Table 8: Canine Plasma/Serum Comparison Study Using The METAT\*\*

Subject	METAT Results* (Plasma/Serum)
83	-/-
85	-/-
86	-/-
88	-/-
90	-/-
93	-/-
96	+/+
102	-/-
104	-/-
112	-/-
113	-/-
114	+/+
118	-/-
119	+/+
127	-/-
128	-/-
130	-/-
132	-/-
141	-/+
142	-/-

\* 1:50 dilution

\*\*2-mercaptoethanol tube agglutination test

Table 9: Agar Gel Immunodiffusion (AGID) Test Results On Canine Sera  
Tested By The METAT<sup>\*\*\*</sup>

Subject/Source	METAT Results	AGID Results
+ Reference Serum	+ 1:3200	Positive + Spur
- Reference Serum	-	Negative
#5/OSU <sup>*</sup>	+ 1:50	Negative
#58/OSU	-	Negative
#62/OSU	-	Negative
#183/OSU	+ 1:400	Positive
#26/Stray <sup>*</sup>	+ 1:200	Partial Identity
#79/Stray	+ 1:400	Positive
#159/Stray	+ 1:200	Partial Identity
#7/V-BDU <sup>*</sup>	+ 1:100	NA <sup>**</sup>

\* OSU=Ohio State University, College of Veterinary Medicine  
Stray=Franklin County Dog Pound  
V-BDU=Vector-Borne Disease Unit, Ohio Department of Health

\*\* not available (insufficient quantity of serum)

\*\*\*2-mercaptoethanol tube agglutination test



Table 10: Rapid Slide Agglutination Test (RSAT) Results On Canine Sera Tested By The METAT\*\*\*

Subject/ Source	METAT Result	RSAT** Result	Subject/ Source	METAT Result	RSAT Result	Subject/ Source	METAT Result	RSAT Result
+ Ref*	+1:3200	+/+	#105/OSU	-	+/+	#48/Stray	+ 1:50	+/+
- Ref	-	-	#106/OSU	-	+/+	#84/Stray	-	+/+
#5/OSU*	+ 1:50	-	#112/OSU	-	-	#109/Stray	-	-
#58/OSU	-	-	#116/OSU	-	-	#151/Stray	-	-
#62/OSU	-	-	#126/OSU	-	-	#175/Stray	-	-
#183/OSU	+ 1:400	+/+	#158/OSU	-	-	#178/Stray	-	-
#26/Stray*	+ 1:200	-	#173/OSU	-	-	#186/Stray	-	-
#79/Stray	+ 1:400	+/+	#178/OSU	-	-	#199/Stray	-	-
#159/Stray	+ 1:200	+/+	#181/OSU	-	-	#13/Dub*	-	-
#7/V-BDU*	+ 1:100	-	#2/Stray	+ 1:50	+/+	#46/Dub	-	-
#26/OSU	-	-	#3/Stray	-	-	#49/Dub	-	-
#76/OSU	-	-	#39/Stray	-	-	#58/Dub	-	-

Table 10: (continued)

Subject/ Source	METAT Result	RSAT** Result	Subject/ Source	METAT Result	RSAT Result	Subject/ Source	METAT Result	RSAT Result
#71/Dub	-	-	#64/AmAd	-	-	#95/Rey	-	-
#9/AmAd*	-	-	#30/Rey*	-	-	#100/Rey	-	+/-
#12/AmAd	-	-	#74/Rey	-	+/-	#1/V-BDU	-	-
#35/AmAd	-	-	#76/Rey	-	-	#12/V-BDU	-	-
#41/AmAd	-	-	#90/Rey	-	-			

\* Ref=reference

OSU=Ohio State University, College of Veterinary Medicine

Stray=Franklin County Dog Pound

V-BDU=Vector-Borne Disease Unit, Ohio Department of Health

Dub=Dublin Veterinary Clinic, Dublin, Ohio

AmAd=American Addition, Columbus, Ohio

Rey=Reynoldsburg Animal Hospital, Reynoldsburg, Ohio

\*\* without 2-mercaptoethanol/with 2-mercaptoethanol

\*\*\* 2-mercaptoethanol tube agglutination test

Table 11: METAT, RSAT, And AGID Test<sup>\*\*</sup> Results

Subject/Source	METAT Results	AGID Results	RSAT Results <sup>***</sup>
+ Reference	+ 1:3200	Positive + Spur	+/-
- Reference	-	Negative	-
#5/OSU <sup>*</sup>	+ 1:50	Negative	-
#58/OSU	-	Negative	-
#62/OSU	-	Negative	-
#183/OSU	+ 1:400	Positive	+/+
#26/Stray <sup>*</sup>	+ 1:200	Partial Identity	-
#79/Stray	+ 1:400	Positive	+/+
#159/Stray	+ 1:200	Partial Identity	+/+
#7/V-BDU <sup>*</sup>	+ 1:100	NA <sup>****</sup>	-

\* OSU=Ohio State University, College of Veterinary Medicine  
 Stray=Franklin County Dog Pound  
 V-BDU=Vector-Borne Disease Unit, Ohio Department of Health

\*\* METAT=2-mercaptoethanol tube agglutination test  
 RSAT=rapid slide agglutination test  
 AGID=agar gel immunodiffusion technique

\*\*\* without 2-mercaptoethanol/with 2-mercaptoethanol

\*\*\*\*not available

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